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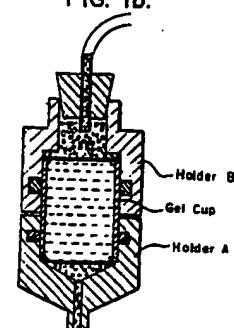
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㉔ A filter and a process for the preparation thereof.

㉕ There is disclosed a filter comprising fibrin in gel form, the gel having substantially uniform pore sizes, and the filter comprising means for retaining the shape of at least one surface of the gel against deformation when contacted by a flowing medium.

FIG. 1b.



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Field of the Invention

This invention relates to a filter with a definite pore size comprising fibrin and a process for the preparation thereof from fibrinogen. This invention also relates to a size selective process employing the filters of the invention.

DISCUSSION OF PRIOR ART

Formation of fibrin gels by contacting fibrinogen with a coagulation enzyme has long been known. It has been observed that when a liquid is passed over the gel, permeation of the gel increasingly becomes difficult - sometimes to the point where permeation and passage of the liquid are rapidly diminished or cease. It was believed that the gel infrastructure was extremely fragile and that the gel consisted of networks of channels and pores of varying size which were highly changeable and highly dependent upon and variable with liquid or liquid mixtures passed thereover, especially one having solid particles.

It was therefore thought that such fibrin gel was not useful in separating components where the separation was effected solely on the basis of particle size.

Specifically, when investigating the fibrin formation from fibrinogen the interest was directed to the flow properties of fibrin gels. It has e.g. been shown before that the flow properties through silica gel as well as through agar and gelatin gels are such as for a viscous flow. It has also been shown earlier that the flow through a fibrin gel is dependent upon the ionic strength and fibrinogen concentration.

in the preparation. In the investigations made, the permeability coefficient (K_s) of the fibrin gels was determined by Poiseulle's law as follows: (Darcy coefficient) $K_s = \frac{Q \cdot L \cdot n}{t \cdot A \cdot \Delta p}$ (1)

wherein Q is the flow through the gel in cm^3 , A is the gel surface in cm^2 , Δp is the pressure difference in dynes/ cm^2 ($=0.1 \text{ N/m}^2 = 0.1 \text{ Pa}$), t is the time in seconds, L is the length of the gel in cm and n is the viscosity in poise ($=0.1 \text{ Pa} \cdot \text{s}$). Moreover, Kozeny-Carman has shown that the following relationship applies in a viscous or laminar flow in a capillary system:

$$m = \frac{K_o \cdot K_s}{\cos^2 \theta \cdot \xi} \quad (2)$$

wherein m is the hydraulic radius ($\frac{\text{wettable surface}}{\text{wettable circumference}}$) in cm, K_o is a factor decided by the geometry of the capillaries, and θ is the orientation (angle) of the capillaries to the direction of flow, ξ is the partial share of liquid in the gel and r is the radius of the capillaries in cm. ξ can be calculated by means of the protein concentration and with a knowledge of the partial specific volume of the fibrinogen which is 0.72. For gels of the type concerned here K_o and $\cos \theta$ cannot be calculated. In the theoretical calculations it has been assumed here that the capillaries are cylindrical and parallel to the direction of flow, which according to Madras et al brings the indicated formula to the following:

$$r = 2m = \sqrt{\frac{8 \cdot K_s}{\xi}} \quad (3)$$

The theoretical pore size is therefore $2r$. By effective pore size we mean: the size at which particles of smaller size pass through the pores and particles of larger size are retained.

It has appeared from the tests that the clotting time (time of gel

1 formation) of the thrombin-fibrinogen mixture, here called Ct, is
2 directly proportional to the flow (Q) through the gel. The
3 flow (Q) has further been found to be inversely proportional
4 to the fibrinogen concentration (C). Provided $Q = 0$, when $l = 0$
5 and $Ct = 0$, the equation (1) will have the following form:
6

$$7 K_s = \frac{k \cdot Ct \cdot K \cdot n}{C \cdot A \cdot P \cdot t} \quad (4)$$

8 wherein k is a constant which is dependent on pH, ionic strength
9 and calcium concentration and, moreover, is characteristic
10 of the enzyme used in the gel formation, and Ct is the clotting
11 time in seconds. The other symbols are the same as in equation
12 (1). The term t is omitted when the flow is expressed in
13 cm^3/s . According to this equation the permeability coefficient
14 K_s is thus directly proportional to the clotting time Ct and
15 inversely proportional to the fibrinogen concentration.
16

17 By varying the pH between 6 and 10 the ionic
18 strength between 0.05 and 0.5, the calcium ion concentration
19 between 0 and 20 mM and/or the concentration of enzyme (e.g.
20 thrombin, "Batroxobin" or "Arvin") between 0.01 and 10 NIH-units
21 (or the corresponding units of other enzymes) per ml solution
22 and the fibrinogen concentration from 0.1 and up to 40 g/l,
23 preferably between 1 and 10 g/l, gels with K_s -values [calculated
24 according to the equation (1)] between 10^{-7} and 10^{-12} , preferably
25 between 10^{-8} and 10^{-11} , can be prepared. Calculated accord-
26 ing to the equation (3), the corresponding average radii will be
27 $0.03 - 9 \mu\text{m}$, preferably $0.09 - 2.8 \mu\text{m}$. If FXIII (a transamida-
28 tion enzyme) and calcium ions are present in the gel formation
29 the stability of the gels will be increased as covalent cross-
30.

1 linkings will arise between the chains in the ~~surfaces of~~ ⁰⁰⁸⁵¹⁶⁶ the
2 gel matrix.

3
4 Thus, now it has been found according to the inven-
5 tion that these fibrin gels can be used as a filter. The
6 filter according to the invention is characterized in that it
7 is built of fibrin and the fibrin gel is in association with a
8 shape-retaining means which retains the shape of at least one
9 surface of said gel against deformation when contacted by a
10 flowing liquid.

11
12 The filter of the invention has substantially
13 uniform pores. By that is meant that the standard deviation
14 of pore size is less than 15 percent, preferably less than
15 10 percent and in some instances less than 5 percent.

16
17 The pore size of the gel has, moreover, been found
18 to be a function of the clotting parameters used in the gels'
19 preparation, i.e., the pore size is varied by changing said
20 parameters. The pore size is then proportional to the clotting
21 time.

22
23 It has now been discovered, in accordance with the
24 invention, that fibrin in gel form can be used as a filter
25 if means are provided to retain the shape of at least one
26 surface of the gel against deformation when the gel is contacted
27 by a flowing medium such as a flowing liquid medium containing
28 components to be separated. It has also been discovered, quite
29 surprisingly, that the gel has substantially uniform pore sizes
30 and that these pore sizes can be regulated simply by altering
31 the process parameters employed for the formation of the gel.

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Specifically, it has been discovered if the gel is in some way stabilized by a shape-retaining means, that the gel structure is preserved and the uniform pores therein function ideally as a filter medium.

Generally speaking, the gel is brought in contact with a shape-retaining means. The shape-retaining means can be a foraminous member such as a foraminous sheet member and is preferably disposed on or in association with an upper surface of the gel, preferably in contact with the gel either directly or through an adhesive or a graft. Since the foraminous member serves to preserve the shape and structure of the upper surface of the gel when the medium to be filtered contacts the same, the gel does not collapse, thereby allowing the uniform pores thereof to function ideally as a filter medium.

Foraminous members functioning as shape-retaining means can have virtually any size and shape, although they are preferably in the form of a sheet and preferably are substantially co-extensive with the upper surface of the gel. The foraminous sheet members can be in the form of a fibrous network such as in the form of a woven or non-woven or knitted fabric, the fibers of which can be natural or synthetic.

When the fibers of a foraminous sheet member are natural, they can be, for example, made of silk, wool, cotton, cellulose, hemp, jute or the like.

As synthetic fibers, there are contemplated in particular fibers made of nylon, polyester, polyolefin, fibers

1 made of vinyl polymers, acrylics such as polyacrylonitrile,
2 rayon, to name a few.

3
4 The fibers generally have a thickness between 1 μm
5 and 1000 μm , preferably between 10 and 20 μm , and are disposed
6 in relationship to one another to define openings therebetween
7 of between 0.01 and 5 mm, preferably between 0.05 and 1 mm,
8 it being understood that the size of the openings between the
9 fibers of the foraminous sheet is not especially critical, pro-
10 vided it allows passage therethrough of the medium to be filtered.
11 It is preferred that as much fiber be in contact with or adhere
12 to the gel as possible so as to insure maximum structural in-
13 tegrity of the surface of the gel initially to come in contact
14 with the medium to be filtered.

15
16 Instead of using a fibrous foraminous member, one
17 can use one made of wires, such as wires made of copper, tin,
18 zinc, aluminum, glass, boron, titanium, steel, stainless steel,
19 etc. The wires function analogously to the function performed
20 by the fibers in providing structural integrity to at least
21 one surface of the gel, preferably the upper surface or sur-
22 face which is to be initially brought in contact with a mixture
23 to be filtered. The interstices between the wires are of the
24 same magnitude as the interstices between the fibers of a woven,
25 non-woven or knitted fabric serving as a foraminous sheet
26 member. The wires can be in the form of a screen, wire mesh
27 or an expanded wire sheet and are preferably co-extensive with
28 at least one side of the gel, preferably the upper surface.

29
30

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The gel has uniform pores but owing to the manner by which the gel can be formed, can have uniform pores over a wide range. Preferably, the substantially uniform pores of the fibrin gel have a theoretical pore size or diameter in the range of about 0.03 to 9 μm , more preferably 0.09 to 2.8 μm .

The gel is formed by contacting fibrinogen with an enzyme, especially a coagulation enzyme. Particularly contemplated enzymes for use in forming a fibrin gel include thrombin, Batroxobin, Arvin, Eccarin, Staphylocoagulase, Papain, Trypsin, caterpillar venom enzyme, etc.

Generally speaking, the gel formation is effected at room temperature, although temperatures from -3°C up to 58°C can be employed. Preferably, the temperature is in the range of 0 to 40°C.

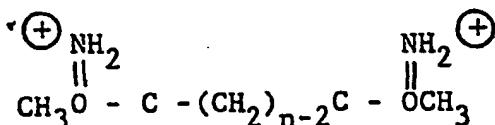
It is preferred that the gel be formed by contacting the fibrinogen with an enzyme in the presence of calcium ions. The calcium ion concentration can be up to 20 mM. The presence of calcium ions is not required in all instances. Where thrombin is employed as the coagulation enzyme, the gel can be formed in the absence of a calcium ion.

In forming the gel, there is generally employed 0.1 to 10^{-5} enzyme units per gram fibrinogen, preferably 10 to 10^{-3} enzyme units per unit weight fibrinogen. Following formation of the gel whose coagulation time is a function of the relative amount of enzyme to fibrinogen as well as the concentration of calcium ion, the gel is preferably hardened or

1 set by crosslinking the components thereof by contacting the
 2 gel with a crosslinking agent. Crosslinking agents contemplated
 3 include bis-imidates such as suberimidate, azides like tartryl
 4 di(ϵ -amino carboxylazide), aryl dihalides like 4,4-difluoro-3,
 5 3'-dinitrophenyl sulfone, glutardialdehyde, nitrenes, N,N'-(4-
 6 azido-2-nitrophenyl)-cystamine dioxide, cupric di(1,10-phenan-
 7 throline), dithio bis-(succinimidyl propionate); N,N'-phenylene
 8 dimaleimide as well as polyethyleneimides and other bifunctional
 9 compounds, especially those known to crosslink with epsilon
 10 lysine, alpha amino groups, carboxy groups of aspartic and
 11 glutamic acids, and hydroxyl groups of amino acids in the pro-
 12 tein chain (e.g. threonine and serine).

13

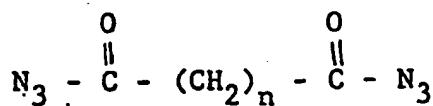
14 Bis-imidates which can be used include those of the
 15 formula



16 wherein n = 3 to 15 especially 3 to 10.

17

18 Azides which can be used include substituted and un-
 19 substituted azides of the formula



20

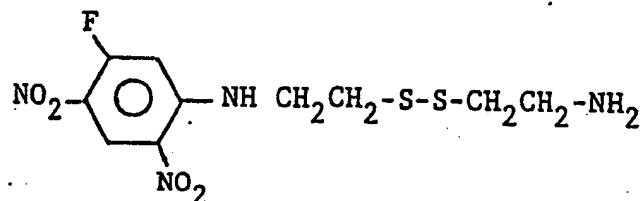
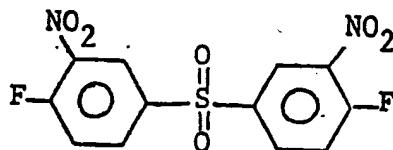
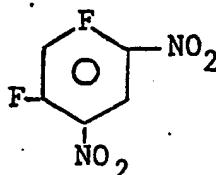
21 wherein n = 1 to 20 especially 1 to 15. Azides contemplated
 22 include those having a hetero atom in the chain, especially ni-
 23 trogen. Also contemplated are hydroxy substituted azides.

24

25 Aryl dihalides which can be used include those
 26 having mono, poly and fused rings as well as rings joined by a

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1 direct bond or through a methylene bridge or a sulfo bridge.
2 The halogen of the halide can be fluorine, chlorine, or bromine.
3 The compounds can be substituted by inert or functional groups
4 such as nitro, or disulfide. Contemplated compounds include
5 those where a functional group has replaced one of the halo
6 substituents, e.g. nitro. Compounds contemplated include



Especially contemplated is glutardialdehyde.

Generally speaking, the crosslinking agent is employed in an amount of between 0.001% and 8 % by weight, preferably between 1 and 2 % by weight of the gel for 1-120 minutes. Crosslinking is effected at temperatures of between 10 and 40°C, preferably 20 to 25°C. After the hardened or crosslinked structure is obtained, the gel is usually washed free of extraneous material.

The gel in such hardened form is useful as a filter, i.e., without any foraminous sheet material. Preferably, however, the gel is formed on or in association with a shape-

1
2 retaining means such as a net, wire mesh or other sheet material
3 and while in contact with such shape-retaining means is hardened
4 by the use of a hardening or crosslinking agent.

5
6 Preferably, the gel is supported on its upper and
7 lower surfaces by a shape-retaining means such as a foraminous
8 sheet or the like, whereby to insure that the gel retains its
9 shape during use as a filter.

10
11 This invention further contemplates a process for
12 separating a first substance having a theoretical size of 0.03
13 to 9 μ m from a second substance having a larger size which com-
14 prises passing a mixture of said first and second substances
15 over a filter comprising fibrin in gel form and having pores of
16 substantially uniform size, said filter having means for retain-
17 ing the shape of at least one surface of said gel against de-
18 formation when contacted by a flowing medium, wherein the ef-
19 fective pore size of said fibrin gel is larger than the particle
20 size of said first substance and smaller than the particle size
21 of said second substance. Preferably, the pores of the gel
22 have a theoretical size of 0.09 to 2.8 mm.

23
24 The filters of the invention are important, as they
25 permit the separation of bacteria and viruses from mixtures con-
26 taining the same. The ability to regulate the pore size and
27 to achieve a gel of uniform pore size is an important and criti-
28 cal characteristic of the filters of the invention. These filt-
29 ers permit the separation of blood components, the separation of
30 components of blood plasma, the removal of platelets from blood,
31 the fractionation of cells and cell fragments and the separa-
32 tion of high molecular weight protein aggregates. In addition,

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1 a variety of particles such as latex, silica, carbon and metallic
 2 particles may be separated over these filters. Components
 3 which can be separated include those shown in the table below:

5 TABLE A

7 Material A 8 Separated	9 Material from 10 Which Material 11 "A" is Separated	12 How Separated	13 Retained	14 Eluted	15 Effective 16 Pore Size 17 Range for 18 Filter
10 Blood platelets	11 Blood plasma	12 X			19 Below 1 μm
11 Red blood cells	12 " "	13 X			20 1 μm and below
12 Sendai virus	13 Culture medium	14 X			21 0.1 μm and 22 below
13 " "	14 " "	15 X	16 X		23 0.2 μm and 24 above
15 Liver mito- 16 chondria	17 Cyto plasma	18 X	19 X		25 0.5 μm and 26 below
17 " "	18 " "	19 X	20 X		27 0.5 μm and 28 above
18 Adeno virus	19 Culture medium	20 X	21 X		29 0.05 μm and 30 below
20 " "	21 " "	22 X	23 X		32 0.1 μm and 33 above
21 E. coli bacteria	22 " "	23 X	24 X		35 1 μm and 36 below
23 FVIII complex	24 High molecular 25 weight material (h.m.w) separ- 26 ated from low mo- 27 lecular weight ma- 28 terial (l.m.w)	29 X (h.m.w)	30 X (l.m.w)		33 0.05 μm and 34 below
25 Blood leucocytes	26 Blood plasma	27 X			36 1 μm and 37 below
27 Blood lymphocytes	28 " "	29 X			39 1 μm and 40 below

1 Fibrin gel filters have above all the advantages
2 over other gel filters that the pore size can be simply varied
3 as desired. Moreover, the present filters have high flow rates
4 at such pore sizes as can be used to remove very small particles,
5 such as virus particles. In this respect, the filters of the
6 invention are more suitable than known membrane filters and
7 filters of polyacrylamide gels. The absence of absorption of
8 protein on the filters is also an advantage as compared with
9 certain other filters.

10

11

DESCRIPTION OF SPECIFIC EMBODIMENTS

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The process according to the invention for the preparation of gel filters is as described above, characterized in that a fibrinogen solution with readjusting clotting parameters is mixed with a coagulation enzyme and the resulting mixture is made to clot in a form intended for the filter. It may be convenient to strengthen the fibrin gel formed during or after clotting by a shape-retaining means of greater strength than the gel which is preferably applied to the upper surface of the gel to be prepared and preferably to both the upper and lower surfaces thereof.

The shape-retaining means (reinforcing meshes) are preferably in the form of a net which is applied at the lower and preferably also at the upper surface in the mold in which the fibrinogen mixture is poured (cast). This mixture can preferably penetrate the net (foraminous sheet material) e.g. have its surface 10 μm -5mm, preferably 0.5-2 mm, from the net. The net can have a mesh width, for example, 10 μm to 5 mm, preferably 50 μm -1mm and the wire diameter can be, for example, 0.01-1.0 mm, preferably 0.1-0.5 mm, where wires are employed as a shape-retaining means. In addition to the metallic wires noted

1 above, wires of natural fibers and plastics can also be employed.
2 The filter can also be reinforced in other places than at the
3 surfaces. It can, for example, be built on a foam material,
4 such as a plastic foam, which can support part of the entire fil-
5 ter.

6

7 The filters of the invention, especially in a non-
8 hardened or crosslinked form, should not be subjected to tempera-
9 tures in excess of 100°C, as such heat sterilization tends to
10 destroy the gel structure. It is, therefore, necessary in
11 utilizing the filters for biological processes to prepare them
12 sterilely from the beginning. On the other hand, it has been
13 found possible to harden or crosslink the filter during the prep-
14 aration by carrying out the gel formation in the presence of
15 Factor FXIII and calcium ions. Where Factor FXIII is to be pres-
16 ent, it is preferably present in an amount of at least 5 units
17 per gram fibrinogen, preferably at least 50 units per gram.
18 Calcium is present in a concentration of at least 20 mM.
19

20 A still stronger filter is obtained by effecting
21 crosslinking with one of the above-mentioned crosslinking agents,
22 especially a dialdehyde and particularly one of the formula
23 OCH-R-CHO, wherein R is an alkylene group of 1 to 8 carbon at-
24oms, such as glutardialdehyde. The filter obtained in this
25 way can be heat treated in an autoclave and consequently steril-
26 ized.
27

28 The clotting parameters are above all the enzyme
29 concentration, e.g. between 100 and 3000 NIH units/L for throm-
30

1 bin and for the fibrinogen concentration between 0.1 and 70 g/l,
2 preferably between 1 and 10 g/l, increased concentration giving
3 a tighter gel. A tighter gel has a smaller pore size. Increased
4 ionic strength also provides a tighter gel as well as a higher
5 pH. It is preferred to carry out the gel preparation using a
6 gel mixture having a pH of between 5.5 and 11, preferably be-
7 tween 6 and 9, and an ionic strength between 0.05 and 0.5. Gels
8 formed at calcium ion concentrations between 0 and 20 mM are
9 tighter.

10

11 Pore size is also affected by the temperature at
12 which the clotting (gelation) is effected. A lower temperature
13 of gelation means an increased clotting time, which in turn
14 means that the resultant gel has a larger pore size. As a re-
15 sult of its larger pore size, it provides a greater rate of flow.
16

17

18 The gel of the invention can be used other than
19 as a filter. One can dispose catalytically active substances
20 such as catalytically active enzymes or catalytically active
21 metals within the pores and thus use the pores' structure as
22 a catalyst. The filter, therefore, can act more or less as a
23 catalyst support for the catalytically active agent disposed
24 therein. When the catalytically active agent is disposed within
25 the pores, the resultant structure can be employed as a size
26 selective catalyst converting only those components whose size
27 is such as to freely pass through the pores of the catalyst
28 support. Those materials retained in the surface of the gel are
29 not catalytically converted.

30

31

32 By such a filter, one can conveniently effect en-
33 zymatic conversions, especially when the enzyme is immobilized

1 within the filter covalently, ionically or otherwise. Since
2 the gel structure is formed by the use of an enzyme, the filter
3 of the invention's chemical components is compatible with the
4 enzyme being employed as an enzyme catalyst. Thus, one can
5 use the filter of the invention for any of the following enzyme
6 conversions when the same contains the appropriate enzyme to ef-
7 fect that enzymatic catalysis: for reactions involving various
8 oxido-reductases, transferases, hydrolases, lyases, isomerases
9 and ligasis (synthetases). Hydrolases which have capacity of
10 degrading the protein strands in the gels cannot be used.
11

12 The method by which the enzyme or other catalytic
13 component is disposed within the filter, i.e., within the pores
14 of the filter, depends upon the nature of the enzyme. Prefer-
15 ably it is disposed by the use of a known enzyme immobilizing
16 agent followed by washing of the filter to remove extraneous
17 materials.
18

19 One can also dispose reactive cellulose components
20 within the pores of the gels. Upon reaction, low molecular
21 weight components may be released and subsequently eluted from
22 the gels. An example of such a type of reaction is production
23 of interferon by leukocytes after their reaction with Sendai
24 virus. As shown in this invention, both of these components
25 can be disposed within the pores of the gels.
26

BRIEF DESCRIPTION OF DRAWINGS

1
2
3 The invention is described more in detail with
4 reference to the enclosed drawing, in which:

5
6 Fig. 1 a shows molding of a filter according to the
7 invention;

8
9 Fig. 1 b shows the filter arranged for filtering;

10
11 Figs. 2 a and 2 b show graphs of the flow as a function
12 of the coagulation time at different pH with thrombin and
13 Batroxobin, respectively;

14
15 Fig. 3 shows the turbidity of the fibrinogen solution
16 (fibrinogen) as a function of the time after addition of thrombin;

17
18 Figs. 4 a and 4 b show the flow as a function of the
19 coagulation time at different ionic strength of thrombin and
20 Batroxobin, respectively;

21
22 Figs. 5 a and 5 b are graphs showing the relationship
23 between protein concentration in the gel forming system and
24 flow-rate.

25
26 Figs. 6 a and 6 b show the temperature plotted against
27 the coagulation time and the flow, respectively, as a function
28 of the coagulation time at different temperatures;

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1 Fig. 7 shows the turbidity of the effluent (turbidity
2 in the effluent) in % as a function of the pore diameter in μm ;
3

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5 Figs. 8 a, 8 b and 8 c are graphs plotting activation
6 of fibrinogen and clotting time (Ct).

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1 In order to more fully illustrate the
2 invention and the manner of practicing the same, the following
3 examples are presented.

4

5 EXAMPLE 16 METHODS AND MATERIALS

7 Human fibrinogen , Fraction 1-4 (7) was obtained from IMCO,
8 Stockholm, Sweden. The preparation, either a freeze-dried powder
9 or a wet paste, was 97-100% clottable (as determined spectro-
10 photometrically). A solution being 0.3 M in NaCl and 2% in
11 protein was prepared. This solution (50ml) was dialyzed against
12 0.3 M NaCl at 4° for 3 hrs. with changes of outer fluid (5 litre)
13 every hour. This dialyzed solution was further diluted with
14 deareated Tris-imidazole buffer (8) of pH between 6.5 and 8.2 to
15 protein concentrations between 1.2 and 5.0 g/l. In the final
16 dilutions the concentration of each Tris and imidazole was 0.02
17 M. When necessary increase in ionic strength was achieved by
18 inclusion of sodium chloride in the buffer. In order to inhibit
19 any trace of plasmin which may be generated, Trasylol (Bayer AG,
20 Germany) was added to a concentration of 5 KIE/ml to all buffers
21 and dialysis fluids.

22

23 In the gelation experiments the following procedures was
24 employed: To 3.65 ml of fibrinogen solution in a plastic tube
25 was added 70 μ l of 1 M CaCl_2 solution, immediately followed by
26 50 μ l of thrombin or Batroxobin solutions of varying concen-
27 trations. This mixture is called Reaction Mixture. The tubes
28 are rapidly inverted twice and transferred to the gel cup or to
29 the spectrophotometer cell within 10 seconds after addition of
30 enzyme. The further handling is described under separate
paragraphs.

1 Thrombin. In most experiments a bovine preparation prepared as
2 previously described (9) was used. Specific activity: 100-200
3 NIH units per mg. Control experiments with highly purified
4 (specific activity: about 2000 NIH units per mg human thrombin
5 (10) was performed in some instances.

6 Batroxobin (from Bothrops marajoensis) was obtained from
7 Pentapharm AG, Basel, Switzerland. Specific activity: 505 BU
8 per mg.

9 Hirudin was also obtained from Pentapharm AG. Specific activity:
10 1000 ATU per mg.

11 Reagents. All reagents used were of analytical grade.
12

13 PREPARATION OF GEL COLUMN

14 A solution of thrombin is added to a solution of fibrinogen
15 in a tris-imidazole buffer containing calcium salts with a pH of
16 between 6.5 and 8.2 and an ionic strength between 0.1 and 0.3
17 so that the final concentration is between 0.05 and 2.5 NIH-
18 units per ml. In other tests Batroxobin is used to obtain gel
19 formation in a concentration between 0.27 and 3.6 BU per ml.
20 The concentration of "Tris" and imidazole salts is each 0.02 M
21 and the concentration of calcium salt is also 0.02 M. The var-
22 iation in ionic strength is obtained by addition of NaCl.
23

24 Gels are also prepared at calcium ion concentration between
25 0 and 20 mM. With reduced calcium ion concentration the opacity
26 of the gels is increased. When thrombin is used to achieve the
27 gel formation the clotting time (C_t), also in the absence of
28 calcium ions is directly proportional to the flow rate and thus
29 also to K_s. When "Batroxobin" is used for the gel formation, the
30

1 stability of the gels in the absence of calcium ions is unsatisfactory, which makes flow measurements more difficult.

2 After addition of an enzyme such as thrombin or Batroxobin,
3 the solution is rapidly mixed and then poured into a cup, e.g.,
4 such a one shown in Fig. 1a. It is made of acrylic plastic^{x)} and
5 has an inside diameter of about 14 mm and a height of about 27
6 mm. The plastic cup is shown in Fig. 1a and the lower part of
7 the cup is provided with a nylon filter having a mesh size of
8 80 x 80 μ m. This filter is fastened by a plastic guard
9 ring. A film layer, e.g., "Parafilm ^Q", is preferably applied
10 at the lower portion so that liquid is prevented from leaking
11 out of the cup. Immediately after introducing the solution
12 into the cup, a silk net with the mesh size 150 x 180 μ m is
13 adapted at the upper end and is fastened with a guard ring. The
14 liquid in the gel cup can e.g., be about 1 mm over the net
15 surface. The cup is left at room temperature for at least 2
16 hours for complete gel formation, preferably in a place free of
17 vibrations.

18 After this time, the film is removed at the lower portion
19 of the cup with its contents of gel is placed in the holder A
20 according to Fig. 1b, a holder B is applied over the upper end
21 of the cup 1. At the upper end of the holder B there is an
22 opening as well as at the lower end of the holder A. The
23 holder B is filled with liquid (buffer or water) and a rubber
24 cork provided with a tube, which is connected with a rubber hose
25 is inserted into the opening. The rubber hose is connected with
26 a container for permeation solution which is allowed to fill the
27 rubber hose without air bubbles. The container (not shown in
28 the drawing) is placed at such a height that a suitable flow is
29

30
x) (other materials can also be used such as nylon and polystyren)

1 obtained through the gel. The hydrostatic pressure is varied at
2 different tests between 4 and 40×10^3 dynes/cm².

3 The fibrinogen used for the preparation of the gels contains
4 trace amounts of factor XIII, which is a transamidase. In the
5 presence of this enzyme and calcium ions, covalent intermole-
6 cular cross linkages between chains in the molecule units of
7 the fibrin gel are formed. This is especially the case when
8 thrombin is present as thrombin activates factor XIII.

9 An electrophoretic analysis of reduced fibrin from various
10 gels in the presence of sodium-dodecyl sulphate shows that a
11 complete cross-linking of the α and γ -chains of the fibrin
12 takes place in the presence of thrombin. A partial cross-linking
13 takes place in the presence of Batroxobin. The covalent
14 cross-linkings formed in the presence of factor XIII contributes
15 to the stabilization of the gel structure.

16 The silk net applied to the top of the gel cup and which
17 is in intimate contact with the gel matrix is of great impor-
18 tance for the mechanical stability of the gels. Without this
19 net or some other means for preventing collapse of the gel, the
20 gel compound is destroyed in the flow tests, the gel collapsing
21 in the central portion and a conical inward bend arising.

22 The silk net can, of course, be replaced with other nets,
23 e.g., of cotton, nylon, iron or copper, which also stabilize
24 the gel structure at pressures up to 40×10^3 dynes/cm².

25 Turbidity measurements

27 In parallel to the flow studies, the turbidity profile of
28 the system was determined under identical conditions. In these
29 experiments the Reaction Mixture (see under Fibrinogen) was
30

1 poured into a cuvette (5ml) of a recording spectrophotometer
2 (Beckman Acta III) and the turbidity (optical density) recorded
3 at 450 nm. After a lag-phase there was a rapid increase in tur-
4 bidity (cf. Fig. 8) which was accompanied by gelation. A
5 tangent was drawn to the steepest part of the sigmoidal curve.
6 Its intersection with the time axis is defined as the gelation
7 or clotting time (C_t). (C_t is about the same as the time for
8 visually observed turbidity increase in the gel cup.) In addit-
9 ion to C_t also maximum turbidity (OD-max) and rate of turbidity
10 increase (Δ OD/min) was recorded. The time required for
11 gelation to reach completion was judged from the turbidity
12 curve. This time ranged from 1 hr. to 2 hrs. for the high and
13 the low enzyme concentrations, respectively.

14 Determination of fibrinopeptides and cross-linking

15 Reaction Mixtures (see under Fibrinogen) were prepared in
16 several identical tubes. One of them was used for turbidity
17 measurement as described above. The other tubes contained each
18 1 ml of Reaction Mixture. The reaction in the latter tubes was
19 quenched at different times by addition of hirudin (2 ATU/ml)
20 and an equal volume of 8 M urea. Thereafter the fibrin (ogen)
21 was precipitated by addition of an equal volume of chilled
22 ethanol. The mixtures were kept on ice-bath for 2 hrs. and
23 thereafter the precipitates were secured by centrifugation,
24 dissolved in urea and used for SDS-gel electrophoresis. The
25 supernatants were used for radioimmunoassay (RIA) of FPA, FPB and
26 B_β 15-42 was assayed using the recently developed method of
27 Kudryk et al.
28

1 Viscosity was determined with a viscometer type Ubbelohde, having
 2 a flow time for water of about 290 sec at 25°C. It was calibra-
 3 ted against a standard (CNI. Cannon Instrument Company, Pa. USA).
 4 Density was determined with a 5 ml pyknometer.

5 Pore size. The equation for calculation of average pore size of
 6 membranes (18) and acrylamide polymer gels (4) was applied:

$$9 \quad r = \sqrt{\frac{8Ks}{\epsilon}} \quad (2)$$

10 where r is the average pore radius (in cm), and ϵ is the fract-
 11 ional void volume of the gel, i.e., the fractional volume of
 12 liquid in the gel. ϵ is calculated on the basis of protein
 13 concentration assuming a partial specific volume for fibrinogen
 14 of 0.72 (19), ϵ is in this case the fractional void volume for
 15 gels in which no water is bound to the gel matrix. However, the
 16 degree of hydration of fibrinogen in solution has been reported
 17 as high as 6 g per g protein.(20). Assuming that this water is
 18 retained by the gel matrix we also calculated ϵ for such
 19 hydrated gels.

20 Diffusion coefficient. The apparent diffusion coefficient of
 21 water in the gel was calculated from Ks according to Ticknor
 22 (21) and White (4).

$$24 \quad D = \frac{R \cdot T \cdot Ks}{\epsilon \cdot V \cdot \eta} \quad (3)$$

25 where D is the diffusion coefficient (in $\text{cm}^2/\text{sec.}$). R is the
 26 gas constant (in ergs/mole-degree), T is the absolute temperature
 27 (in K) and V is the molar volume of the permeant (in cm^3/mole).
 28

1 Ionic strengths were calculated on basis of the molarity of the
2 electrolytes. Activity coefficients and degree of calcium
3 binding to protein were not taken into account.

4 Least square analysis was used for calculation of correlation
5 coefficients, slopes and intercepts. All lines shown in figures
6 were drawn accordingly.
7

8 : RESULTS

9 Preparation and stability of gels .

10 The flow studies were performed on gels which had been
11 formed at ambient temperature. The average temperature was
12 $24 \pm 2^\circ$. However, in each series of experiments the variation in
13 temperature never exceeded 2° . Preliminary experiments suggested
14 that this variation in temperature has a negligible effect on
15 C_t of the system. In the permeation experiments, when not other-
16 wise stated, the flow-rates were corrected to 25° .
17

18 The silk net at the upper end of the gels stabilizes the
19 gel structures. Without support of the silk net, the gels will
20 yield to flow at the pressure applied (about 7×10^3 dynes / cm^2).
21 The yielding is only noted at the center of the gel, since the
22 gel matrix adhers firmly to the walls of the plastic cup.

23 The nets in the column do not significantly reduce the flow-
24 rate of liquid in columns without gels. We, therefore, assume
25 that also when the nets are in contact with gels they do not
26 restrict the area available for flow.

27 Before a flow experiment was started, the extent of
28 incorporation of fibrinogen into the gel matrix was determined.
29 This was done by determining the protein content in the void

1 volumne of the column (about 4 ml). The amount of protein, as
2 measured spectrophotometrically using the extinction coefficient
3 of fibrinogen (22), ranged between 1 and 3% of the total protein
4 used for gelation. When deemed necessary the non-clottable
5 portion was taken into account in calculations of the fibrin
6 content of gels.

7 The effect of changeing permeant on the flow-rate of gels
8 was studied in some experiments. A representative series of
9 experiments is shown in Table I. The gel was first percolated
10 with buffer of ionic strength 0.21 (experiment I). On changing
11 the permeant to water (experiment II) an increase in flow-rate
12 occurred. which is larger than expected on the basis of the
13 viscosity change of the permeant. On return to the original
14 permeant (experiment III) the flow-rate decreased, but not
15 completely to the original value (experiment I). When buffer of
16 ionic strength 0.36 was percolated through the gel (experiment
17 IV) a small decrease occurred which was almost as expected on the
18 basis of the difference in viscosity between the two buffers.
19 When the permeant was again changed to water (experiment V) the
20 flow-rate increases to almost the same value as after the first
21 change to water (experiment II). These results suggest that the
22 final gel structure is not influenced by moderate changes in
23 permeant composition, but changes may occur on drastic changes
24 in ionic environment and these are not completely reversible.
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1 TABLE I

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2 Flow Properties of Fibrin Gels with Different Permeants.

3 Gel formation: Tris-imidazole buffer pH 7.4, ionic strength
4 0.21, thrombin 0.8 NIH-units/ml, temperature 21° and fibrinogen
5 concentration 2 mg/ml.6 Permeation:* at 22° - 23.5°.

Experiment	Permeant	Flow, ml/hr	%
I	Tris-imidazole pH 7.4, $\bar{I}/2$ 0.21	3.177	100
II	H_2O	3.708	117
III	Tris-imidazole pH 7.4, $\bar{I}/2$ 0.21	3.385	106
IV	Tris-imidazole pH 7.4 $\bar{I}/2$ 0.36	3.271	103
V	H_2O	3.649	115

16 Flow pattern through fibrin gels

18 Viscous flow. In order to test if the flow obeyed Poiseuille's
 19 law, the flow-rate at different pressures ($4.5 - 5.6 \times 10^3$ dyne/
 20 cm^2) for gels formed at pH 7.4, ionic strength 0.21 was deter-
 21 mined, at three different thrombin concentrations (0.1-0.8 NIH
 22 units/ml). The K_s -range for these gels was 10^{-8} to 10^{-10} .
 23 Permeation was in one experiment with the same buffer as above
 24 and in the other cases with water. In all cases the drop-rate
 25 decreased linearly with decreasing pressure. As shown in Table
 26 II for one of the gels, the flow-rate per unit pressure was
 27 almost independent of total pressure.

28 In another series of experiments the flow-rates were de-
 29 termined with permeants of different viscosities. The gels used
 30

1 in these experiments were formed at pH 7.4, ionic strength 0.21,
2 at four fibrinogen concentrations. Thrombin as well as
3 Batroxobin were used as inducers of gel formation. Permeation
4 was performed at five different temperatures between 4.5° and
5 40°. In all cases there was a linear relationship between the
6 inverse viscosity of the permeant and the flow-rate. These
7 experiments suggest that the flow through the gels is viscous.
8 In addition Reynold's number was calculated and found to be
9 within the laminar region for all gels.

10 Diffusive flow. It was pointed out by Ticknor, J. Phys. Chem 62,
11 1483-5 (1958) that the equation for viscous flow is identical
12 in form to equations for diffusive flow, when the relationship
13 between diffusion coefficient (D) and viscosity according to Johnson and
14 Babb, Chem. Revs. 56, 387-453 (1956) is taken into consideration.
15 The relation between K_s and D is given in Equation 3. In flow
16 experiments using water as permeant we calculated the apparent
17 diffusion coefficient for water at 22° - 23°. Even for the
18 tightest gels ($K_s 10^{-10}$), the calculated D-values were 6-orders
19 of magnitude larger than the reported self-diffusion coefficient
20 of water at 25° ($2.8 \times 10^{-5} \text{ cm}^2/\text{sec.}$). This supports the above
21 conclusion that the flow through the fibrin gels is predominately
22 viscous.

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1 TABLE II

2 Relationship Between Pressure and Flow-Rate.

3 Gel formation: pH 7.4, ionic strength 21, thrombin 0.1 NIH unit/ml
4 temperature 23.5° and fibrinogen concentration 2 mg/ml.5 Permeation: H₂O, temperature 23.5°. K_s = 9 × 10⁻⁹

7 Pressure, 8 dyne/cm ²	7 Flow 8 ml/hr	7 Flow per dyne per cm ² 8 ml/hr × 10 ³	7 %
5531	100	2.0394	100
5319	96.2	2.0337	99.7
5127	92.7	2.0320	99.6
4874	88.1	2.0228	99.2
4576	82.1	1.9991	98.0

15 Gel Permeability and clotting time (C_t)16 There is a correlation between clotting time (C_t) of
17 fibrinogen and enzyme concentration. We explored the
18 relationship between C_t and permeability of the final gels.
19 Therefore, at the same time as gels were prepared for permeabil-
20 ity studies, the C_t of the gel forming system was determined in
21 parallel experiments by turbidity measurements (see Methods).
2223 pH. At a constant fibrinogen concentration and ionic
24 strength, the flow-rates for both thrombin and Batroxobin gels
25 were directly related to the C_t of the gel forming system over a
26 wide range of C_t (17 sec - 500 sec). This applied to three
27 different pH's (6.5, 7.4 and 8.2) as exemplified in Fig. 2. At
28 all pH's there was a difference in slope between curves for
29 thrombin as compared to those for Batroxobin.

1 At each pH, the correlation coefficients (r) for six diff-
2 erent Ct versus flow-rate curves (4 experimental points in each)
3 were calculated. The mean r-values and their standard deviations
4 (SD) were as follows: at pH 6.5, 0.9709 ± 0.0184 ; at pH 7.4,
5 0.9721 ± 0.0394 ; at pH 8.2, 0.9599 ± 0.0434 . There was no sig-
6 nificant difference in r-values for thrombin and Batroxobin
7 curves.

8 Ionic strength. In another series of experiments the ionic
9 strength of the gel forming system was, at constant protein con-
10 centration, varied between 0.21 and 0.31. At all pH's (6.5, 7.4,
11 8.2) an increase in ionic strength from 0.2 to 0.3 resulted in a
12 decrease in flow-rate by roughly one order of magnitude. This
13 applied to both thrombin and Batroxobin gels.

14 Ct were prolonged with increasing ionic strength at all
15 enzyme concentrations and pH's. At each ionic strength, however,
16 there was for both thrombin and Batroxobin gels a linear re-
17 lationship between Ct and flow-rate. The results at pH 7.4 is
18 shown in Fig. 4. At all ionic strengths a difference in slope
19 between curves for thrombin as compared to those for Batroxobin
20 was noted.

21 At two ionic strengths, regardless of pH, r-values for six
22 different Ct versus flow-rate curves (4 experimental points in
23 each) were calculated. Mean r-values and SD were as follows: at
24 ionic strength 0.21, 0.9851 ± 0.0208 and at ionic strength 0.26,
25 0.9511 ± 0.0470 . There was no significant difference in r-values
26 for thrombin and Batroxobin curves.

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1 Gel permeability and fibrinogen concentration

2 In a series of experiments we showed that the relationship
3 between Ct and flow-rate applied to a wide range of protein con-
4 centrations in the gel forming system. These experiments were
5 only performed at pH 7.4 and ionic strength 0.21. When Ct at a
6 given protein concentration were plotted against flow-rates a
7 linear relationship, similar to that shown in Fig. 3 (pH 7.4),
8 was demonstrated at all fibrinogen concentrations (1.5 - 5.0 g/l).
9 The plots for both thrombin and Batroxobin converged towards an
10 intercept near the origin with decreasing closing times. Like
11 in the experiments shown in Fig. 2, the slopes for Batroxobin
12 curves were steeper than those for thrombin at all protein con-
13 centrations. The r-values for 8 different Ct versus flow-rate
14 curves (8 points in each) were calculated. Mean r-values and
15 SD were as follows: for thrombin, 0.9800 ± 0.0157 and for
16 Batroxobin, 0.9830 ± 0.0187 .

17 Table III shows Ct at different protein and enzyme con-
18 cnetrations in one series of experiments. In case of Batroxobin,
19 increasing protein concentrations did not markedly influence Ct.
20 However, in the case of thrombin there is a small prolongation
21 of Ct with increasing fibrinogen concentrations.

22 The relationship between protein concentration in the gel
23 forming system and flow-rate was next studied. Fig. 5 shows the
24 result of one series of experiments. It is evident that there
25 exists, at different enzyme concentrations, a linear relationship
26 between flow-rate and inverse protein concentration. The curves
27 from thrombin and Batroxobin gels converge to a more or less
28 common intercept near the origin with increasing protein con-
29

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1 concentration. The r-values for 15 different l/C versus flow-rate
2 curves (4-8 experimental points in each) were calculated. Mean
3 r-values and SD were as follows: for thrombin, 0.9738 ± 0.0308
4 and for Batroxobin, 0.9711 ± 0.0356 .

5

6 It is apparent from Fig. 2 how the flow rate $\frac{Q}{t}$, at a con-
7 stant hydrostatic pressure, is directly proportional to the
8 coagulation time (Ct) of the thrombin-fibrinogen mixture at
9 different pH. The coagulation time of the system is determined
10 spectrophotometrically in a separate test under otherwise ident-
11 ical conditions. The optical density OD at 450 nm is determined.
12 At gel formation the turbidity of the solution increases rapidly
13 as shown in Fig. 3. The tangent of the steepest portion of the
14 curve intersects the time axis at a distance designated as
15 coagulation time Ct. As there is a direct relation between Ct
16 and flow rate $\frac{Q}{t}$, Ks is also directly correlated with Ct accord-
17 ing to equation 1.

18

19 It is apparent from Fig. 4 that the flow rate of gels formed
20 at different ionic strength is always directly correlated to the
21 Ct of the enzyme-fibrinogen solution used in the gel preparation.
22 In addition the great influence on the flow rate at a change of
23 the ionic strength is pointed out.

24

25 As is apparent from Fig. 5, the flow rate $\frac{Q}{t}$ is inversely
26 proportional to the fibrin concentration (fibrinogen concentra-
27 tion) in the gel. Thus, according to equation 1, Ks will also
28 be inversely proportional to the fibrinogen concentration.

29

30 The flow is dependent on the temperature, as according to
equation 1, the flow is inversely proportional to the viscosity
of the permeation solution. The temperature in gel formation is

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1 also of importance as a constant enzyme concentration, Ct is
2 reduced at a higher temperature. This is apparent from Fig 6a.
3 However, the flow rate in gels formed at different temperatures
4 is directly proportional at Ct at the relative temperature, as is
5 evident from Fig. 6b.

6 The columns prepared in the way schematically illustrated
7 in Fig. 1 are of small dimensions ($1.5 \text{ cm}^2 \times 2.6 \text{ cm}$). Similar
8 qualitative results are observed with gel columns of greater
9 dimensions ($5 \text{ cm}^2 \times 12 \text{ cm}$). When nothing else is indicated, the
10 smaller type of column is used in the tests.

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3 Standardization of pore size with latex particles of a known
4 size.5 Spherical latex particles of diameters between $0.085 \pm$
6 0.0055 (SD) μm (SD = standard deviation) and 0.198 ± 0.0036 (SD)
7 μm from Dow Chemicals, USA, were used in the tests. A number of
8 gels formed at pH 7.4 and at two different ionic strengths, were
9 used. In the tests, C_t varied from 23 to 314 sec-
10 onds. The theoretical radius was calculated for each gel according
11 to equation 3 assuming that a cylindrical vertical capillary
12 system was present.13 Fig. 7 shows two series of tests. In series I the ionic
14 strength was 0.23 during the gel formation and in series II
15 0.21. The gel columns were equilibrated with water. After this
16 suspensions of particles were applied to the gels. In series I
17 the particle size was $0.085 \mu\text{m}$ and in series II $0.198 \mu\text{m}$. The
18 particles were slurried in water to a concentration of 0.1%
19 (weight/volume). The turbidity at (450nm) of the effluent was de-
20 termined. It was then possible to establish by means of the
21 turbidity values if the latex particles had passed the filter.
2223 In Fig. 7 the turbidity has been expressed in % of maximum
24 turbidity of the effluent. As is apparent from Fig. 7 the
25 turbidity of the effluent increases above a certain theoretical
26 pore size of the gel. At an additional increase in pore size
27 more particles will pass through the gel (filter) and over a
28 certain pore size a constant amount of particles permeate the
29 gels. The difference in theoretical pore size between no and
30

1 complete permeation is a measure of the sum of pores and
2 particle variation. The pore size at 50% permeation is an ex-
3 pression of the average size of pores and particles. If the
4 total variation in pore size is within the range of the average
5 particle size \pm 3 SD it can be assumed that the pore size in the
6 gel is uniform.

7 In Fig. 7 the variation in particle size (average size \pm 3
8 SD) has been shown with a horizontal line 50% permeation. It is
9 apparent that the total variation can, to a large extent, be ex-
10 plained by the particle variation. It can be concluded from this
11 that the pores in the gels are rather uniform. It is also
12 apparent from Fig. 7 that the theoretical average pore size is
13 about of an order (one ten power) greater than the real effective
14 particle size. Thus, calculation of the pore size according to
15 equation 3 only gives relative values for the pore size.
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1 EXAMPLE 33 Passage of proteins and dextran through fibrin gels

5 Various protein solutions were (applied) to fibrin gels pre-
6 pared in the way described in Example 1. The gel formation was
7 carried out at room temperature (21 - 25°C). In most cases, the
8 buffer used in gel formation had the same composition as the
9 buffer used for permeation. The filtration tests took
10 place at room temperature (22 - 25°C). When nothing else is in-
11 dicated, the volume of the gels was $1.47 \text{ cm}^2 \times 2.48 \text{ cm} = 3.65 \text{ ml}$.
12 The tests were carried out on different days and with different
13 fibrinogen preparations. Thus, it is not possible to make a
14 comparison as to the pore size between different tests. Table 1
15 shows the proteins tested with respect to filtering ability
16 through fibrin gels of different porosities. It is apparent
17 from the table, that proteins, including those having a very
18 high molecular weight, are filtered even through gels having
19 small pore sizes. A high molecular weight polysaccharide ("Blue
20 Dextran") shows the same filtration properties as the proteins.
21 The table shows that the yield of proteins in the eluate is high,
22 from which it appears that at least at room temperature the
23 interaction between gel matrix and proteins is small.
24 This also applied to such proteins as fibrinogen, fibronectin
25 and the factor VIII complex.

TABLE I
Filtration of proteins and particles through fibrin gels

Test	Diameter or mol. weight	Permeation Buffer, ml	Sample Yield, ml	Pore diam. μm	Gel formation			Enzyme
					Ct s	Protein g/l	$\frac{P}{2}$	
Human serum	-	Tl buffert + Ca ²⁺	0.1	98.2 (0,125-0,286)	2,12-4,86	16-230	3,42	0,21
Human plasma	-	Tl buffert -Ca ²⁺	0,1	101.1	0,288	260	4,56	0,26
Fibrinogen (4g/1)	340000	Tl buffert -Ca ²⁺	1,0	93,8 (0,255)	4,34 (0,137)	124	2,157	0,21
Fibrinogen (4g/1)	340000	Tl buffert +Ca ²⁺	1,0	97,8 (0,137)	2,33	66	2,157	0,21
Fibronectin (2,3g/1)	440000	Tl buffert -Ca ²⁺	0,4	10,9 (0,135)	2,30	430	2,274	0,21
Antihämophilic factor (FVIII) b	$2 \cdot 6 \times 10^6$	Tl buffert +Ca ²⁺	1,0	99,8 (0,146)	2,48 1,08 (0,064)	3,000	0,21	7,40
Ferritin (15g/1)	2×10^6	Tl buffert +Ca ²⁺	0,2	103,8 (0,016)	0,276	440	4,56	0,26

a) The theoretical diameter was calculated according to equation 3. The effective diameter through calibration with latex particles.

The effective pore diameter is given within brackets. (Based on a ratio $\frac{\text{theoretical}}{\text{effective}}$ of about 176)

b) The volumes of the gel column; $4,9 \text{ cm} \times 11 \text{ cm} = 53,9 \text{ ml}$.

TABLE 1 (cont.)

Test	Diameter or mol. weight	Permeation Buffer	Sample ml	Yield %	Pore diam. μm	a) Ct s	Gel formation Protein g/l	$\frac{n}{2}$	pH	Enzym
Hiroya-nin (11g/l)	3×10^6	TT buffer + Ca^{2+}	0.2	97.7	0.276 (0.016)	440	4.56	0.26	7.40	T
Human red blood corpuscles $5 \times 10^6/\mu\text{l}$	7 μm	Physiological common salt solution - Ca^{2+}	0.1	0	3.60 (0.212)	255	1.66	0.21	7.40	T
Human Platelets $(4 \times 10^5/\mu\text{l}$ plasma)	2 \sim 4 μm	TT buffer - Ca^{2+} + 10m M EDTA	0.3-5	0	4.86 (0.286)	228	3.42	0.21	7.40	T
Rat liver mitochondria	0.5 μm	TT buffer - Ca^{2+} + 0.25M sucros, + 10m M EDTA	0.3	0	4.40 (0.259)	524	2.16	0.21	7.40	T
E. Coli	0.8x1.2 μ	TT-buffer - Ca^{2+}	45	0	1.78 (0.105)	24	5.00	0.21	7.40	T
Sendai-virus (640) hemagglutination units /ml	0.15 μm	TT buffer - Ca^{2+} + 1% BSA	I 0.2 II 0.2 III 0.2	0 50.3 95.2	0.284 (0.017) 2.36 (0.139) 4.46 (0.262)	33 19 137	4.63 2.08 2.08	0.233 0.21 0.21	7.40 7.40 7.40	T T T
Sendai-virus filtered through hardened thin layer fibrin gel (see Example 6 and Table 2-III)	0.15 μm	TT buffer + Ca^{2+}	5	-	0.268 (0.016)	60	9.86	0.26	7.40	T
						0.86				
						85.0				

2
3 Filtration of suspensions of red blood corpuscles through
4 fibrin gels5
6 Fibrin gels were prepared in the way described in Example I
7 and the conditions of gel formation is shown in Example 3. A
8 small amount (0.2 ml) of human blood was applied to a gel column.
9 Continued filtration was carried out at room temperature (22 -
10 25°C) under the conditions shown in Example 3. The blood
11 corpuscles did not pass through the fibrin gel. This was ex-
12 pected as the diameter of the red blood corpuscles (7-8 μ m)
13 is much larger than the effective pore diameter of the fibrin
14 gel.

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1 EXAMPLE 5a

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3 Filtration of plasma rich in platelets through fibrin gels

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5 Plasma rich in platelets (PRP) was prepared from blood by cen-
6 trifugation for 4 minutes at 120g, the blood being drawn in
7 citrate solution to prevent coagulation. It was centrifuged at
8 2000 g for 5 minutes to remove the remaining red blood corpuscles
9 and EDTA at a concentration of 10 mM was added to the PRP.
10 0.5 ml of the PRP was applied to a fibrin gel column prepared in
11 the way described in Example I. The conditions of gel formation
12 is shown in Example 3 and filtration was continued under the
13 conditions shown in Example 3. To prevent aggregation of the
14 platelets and their adhesion to the gel matrix, EDTA (10 mM) was
15 added not only to PRP but also to the solution which was filtered.
16 No platelets could be demonstrated in the eluate from the fibrin
17 gel column. This was expected as the diameter of the platelet
18 lies between 2 and 4 μm , which is considerably more than the
19 effective pore size of the gel.

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2
3 Separation of mitochondria from fragments of liver cell by
4 filtration through fibrin gels5
6 Liver cells of a rat were homogenized in a homogenizator
7 according to Potter-Elvehjem. Separation of cell fragments was
8 achieved by differential centrifugation in known manner. The
9 mitochondria were slurried in a buffer solution containing Na-
10 EDTA (10 mM) and sucrose (0.25M). 0.3 ml of the resulting
11 suspension was applied to a fibrin gel column prepared in the way
12 described in Example 1. The conditions of gel formation is shown
13 in Table I and filtration was continued under the conditions
14 indicated in Table I. No mitochondria could be demonstrated in
15 the eluate, which was as expected, since their diameter is about
16 0.5 μ m; thus considerably bigger than the effective pore size
17 of the gel.18
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2 Separation of Sendai-virus by filtration through fibrin gels3
4
5 Sendai virus is a virus specific to mice which is used for pre-
6 paration of interferon in human lymphocyte cultures. A partially
7 purified virus preparation (640 hemagglutination units/ml) was
8 used in the tests.9
10 0.2 ml of the virus suspension was applied to each of three
11 fibrin gel columns prepared in the way described in Example 1.
12 The conditions of the gel formation appear from Table 1 and
13 filtration was continued under the conditions shown in Table 1.
14 No hemagglutination activity could be demonstrated in the eluate
15 from column I; 50% of hemagglutination activity were demonstrated
16 in the eluate from column II and 95% of the hemagglutination
17 activity of the virus particles was demonstrated in the eluate
18 from column III (see Table 1).19 After filtration the silk nets at the upper part of the three
20 columns were washed with a buffer solution (containing 1 % of
21 bovine serum albumin, BSA) and the hemagglutination activity of
22 the washings was analyzed. In the washing liquid from columns I
23 100% of the hemagglutination activity was found; in the washing
24 liquid from column II 25% of the activity was found and in the
25 washing liquid from column III no activity was found.26 The particle diameter of Sendai virus is stated to be about
27 0.15 μ m. The tests show that when the effective pore radius is
28 more than 0.15 μ m the virus particles pass through the gel. When
29 the effective pore radius of the gel is less than 0.15 μ m a re-
30 tention of the particles will, on the other hand, occur.

1 EXAMPLE 72
3 Separation of Eschericia Coli (E. coli) by filtration through
4 fibrin gel.

5
6 E. coli is an elongatedintestinal bacterium of the approxi-
7 mate dimensions $0.8 \times 1-2 \text{ um}$. A suspension of E. coli in tris-
8 imidazole-buffer, free of calcium and with pH 7.4 and ionic
9 strength 0.21, was prepared (see Example 1). The suspension con-
10 tained between 10^7 and 10^8 bacteria/ml. 45 ml of the suspension
11 were supplied to a fibrin gel column of the dimensions $5 \text{ cm}^2 \times$
12 11 cm prepared in the way described in Example 1. The conditions
13 of the gel formation and the filtration are shown in Table 1.
14 The flow rate was 31 ml/h. No bacteria passed through the gel,
15 determined by turbidity measurements of the eluate from the
16 column. The flow rate at constant pressure was less at the end
17 of the test than at its beginning. Assuming an unchanged K_s
18 the reduction of surface corresponding to the reduction in flow
19 can be calculated according to equation 1. According to this
20 calculation the surface had been reduced to 58%. Thus, one
21 might expect that the bacteria were enriched on the upper gel surface.
22 By washing the silk net attached to the upper part of the gel
23 with buffer solution 99% of the bacteria applied to the gel
24 were found in the washing liquid.

25
26 The test shows that E. coli cannot pass through gels having
27 a pore diameter which is considerably less than the smallest
28 diameter of the bacteria.

2
3 Preparation of gels with reinforcement of porous plastic4
5 In the foregoing examples nets of silk, plastic or metal
6 adapted to the upper and lower portions of the gel have served
7 as stabilizing structure of the fibrin gels. A corresponding
8 stability can also be obtained in such a way that the fibrin gel
9 is cast into a porous plastic, e.g. polyurethane, polyester or
10 some similar porous plastic material, preferably one which is
11 wettable by water.12 In this example a foam plastic of polyurethane ("Regilen 40
13 AG") of a pore size 0.4 mm has been used. The gels were cast in
14 a special apparatus. This consisted of a cylindrical plastic
15 chamber in which the porous plastic had been introduced; the
16 plastic was accommodated in a ring of acrylic plastic (height 2 cm
17 and diameter 9 cm). The apparatus (chamber) had an opening at
18 the upper and lower end, respectively. One opening was connected
19 to a vacuum pump and the other opening was kept closed. The
20 chamber was evacuated by means of the vacuum pump. After this
21 the valve connecting the chamber with the vacuum pump was shut
22 off. A fibrinogen-trombin solution was subsequently allowed to
23 fill the chamber rapidly through the valve in the opposite opening.
24 The valve was thereafter closed and the chamber was left for 2
25 hours, so that the fibrinogen solution in the porous plastic
26 material should be completely converted to a fibrin gel. The
27 clotting parameters of the thrombin-fibrinogen mixture was shown
28 in Table 2. For comparison a gel was also prepared in the way
29 described in Example 1. In Table 2 the Ks -value of this latter
30

1 gel is also shown. After complete gel formation the chamber was
2 opened and the plastic cake with fibrin gel(including its plastic
3 frame) was taken out. It was transferred to a special filter
4 chamber. The framed ring, in which the plastic material and the
5 gel were accomodated, fitted tightly to the edges of the filter
6 chamber through two O-rings. The upper lid of the chamber was
7 provided with an inlet for the liquid to be filterd and a vent-
8 ilating valve to let our the air above the gel surface. In the
9 lower portion of the chamber there was an outlet for collecting
10 the filtered liquid. A buffer solution with the composition
11 shown in Table 2, was filtered through the gel cake. The K_s
12 value was calculated according to equation 1 (Table 2). As is
13 apparent from the table the K_s -value of the gel, cast in plastic,
14 is of the same order as the gel prepared according to Example 1.
15 The partial specific volume of the plastic material in the gel
16 cake is 0.03 which means that the plastic matrix reduces the
17 surface available for flow only to a small extent.
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EXAMPLE 9

Preparation of gels in a cellulose matrix

Cellulose materials can also be used as reinforcing agent (supporting substance). In this example, a porous cellulose compound ("Wehex cloth") is used as reinforcing agent of the fibrin gel. It had a thickness of 0.2 - 0.3 cm. Circular pieces of a radius of about 3 cm were wetted with a thrombin-fibrinogen solution. The cellulose pieces then swelled to about double thickness: The partial specific volume of the swollen cellulose compound was 0.04. Immediately after swelling which lasted for about 2 - 4 seconds the pieces were placed on the filter disc of a Büchner funnel. Measures were taken so that the pieces fitted tightly to the edges of the funnel. The openings of the funnel were covered with "Parafilm" and the funnel was left at room temperature for 2 hours in order to obtain a complete fibrin formation in the pores of the cellulose. Buffer solutions, the composition of which is shown in Table 1, were filtered through the gels. The K_s -value of the gels which are cast in cellulose is of the same order of magnitude as control gels prepared without reinforcing substance.

1 EXAMPLE 102 Preparation of fibrin gels in thin layers for filtration

3 In this example it is shown that fibrin gels in thin layers
4 with reinforcement only on the lower surface can be used for
5 filtration. About 10 ml of fibrinogen solution in tris-
6 imidazole buffer with pH 7.4 were mixed with a thrombin solution.
7 The mixture was thereafter poured into a Petri cup the bottom of
8 which was covered by a damp silk cloth. The cup was covered with
9 a lid and was left for 2 hours for a complete gel formation. The
10 thickness of the gel layer was 2 mm. The clotting parameters of
11 the gel is shown in Table 2. The filter was thereafter attached
12 to a "Millipore" filter support provided with a funnel. The
13 funnel was filled with buffer solution and the flow rate was
14 determined. As is apparent from Table 2 the Ks-value is of the
15 same order or magnitude for a corresponding fibrin gel prepared
16 according to Example 1. However, the filter showed in course of
17 time gradually diminishing Ks-values, which presumably is due to
18 compression of the gel matrix during the flow.

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1 EXAMPLE 11

2

3 Stabilization of gels by treatment with dialdehyde

4

5 In this example it is shown that gels prepared according to
6 Examples 1, 8 and 10 can be stabilized by treatment with
7 dialdehyde.

8 A. A gel prepared according to Example 1 was first equili-
9 brated with water and then brought into equilibrium with 0.014 M
10 phosphate buffer solution with pH 7.2 in 0.15 M NaCl (phosphate
11 buffered saline solution PBA). 2 - 4 column volumes of a 1%
12 glutaraldehyde solution were then allowed to filter through the
13 gel in the course of 10 minutes - 2 hours. After this the gel was
14 washed with several column volumes of PBS and then with water.
15 The column was finally equilibrated with tris-imidazole buffer
16 and flow measurements were carried out. The Ks-value is almost
17 unchanged after treatment with glutar dialdehyde. After the
18 flow measurements, the gel was taken out and treated for 72 hours
19 with 8 M urea containing 1% of sodium dodecyl sulphate (SDS).
20 The gel was then reduced with 1% dithiotreitol in a way known
21 per se. Polyacrylamide gel electrophoresis in the presence of
22 SDS showed in comparison with non-stabilized gels the absence of
23 free fibrin chains (fibrinogen chains), which can be interpreted
24 as a proof that glutar dialdehyde had cross-linked the chain
25 units of the fibrin structure.

26

27 B. A gel prepared in porous plastic according to Example *
28 was first washed with a tris-imidazole buffer solution free of
29 calcium and was then brought into equilibrium with a 0.014 M

30

1 phosphate buffer solution with pH 7.2 in 0.15 M NaCl (PBS).
2 Two column volumes of a 1% glutar dialdehyde solution were then
3 passed through the gel cake (column) in the course of 10 minutes.
4 The gel cake was then washed with several column volumes of PBS
5 and then with water. Finally the column was brought into equili-
6 brium with tris-imidazole buffer and flow measurements were
7 carried out. These are shown in Table 2. As is apparent from
8 the Table the K_s -value is only slightly changed after the treat-
9 ment with glutar dialdehyde and is of the same order of magni-
10 tude as a gel prepared according to Example 1. The gel stabil-
11 ized with glutar dialdehyde was then autoclaved at 120°C for 20
12 minutes at a pressure of 1.4 atm. After autoclaving the flow
13 of buffer solution was again tested through the gel cake. As is
14 apparent from Table 2, autoclaving has influenced the flow prop-
15 erties of the gel only to a small extent. Cracks in the gel.
16 would have caused drastic increase, of the flow through the gel.

17 C. A fibrin gel prepared according to Example 10 was
18 transferred to a cup with 500 ml water to remove buffer salts by
19 diffusion. After 2 hours the gel was transferred to a cup with
20 a new portion of water. After 2 hours the gel was transferred to
21 a cup with 500 ml phosphate buffer solution with pH 7.2 in 0.15
22 M NaCl (PBS) and was left over night. The gel was then trans-
23 ferred to a Petri cup containing 50 ml of 1% glutar dialdehyde.
24 After 2 hours the glutardialdehyde solution was exchanged for a
25 new portion of the same liquid. After additional 2 hours the gel
26 was transferred to a cup with water and washed in the way des-
27 cribed above. After washing with water the gel was transferred
28 to a cup with tris-imidazole buffer solution. After 2 hours the
29 washing liquid was exchanged for a new portion and after addit-

1 ional 12 hours the gel was transferred to a "Millipore"-filter
2 carrier with a funnel. As a comparison measurements were carried
3 out with a gel prepared in the same way except the treatment with
4 glutar dialdehyde. Directly after casting, this gel was trans-
5 ferred to a "Millipore" filter container for flow measurements.
6 As is apparent from Table 2 the flow through the vulcanized
7 filter was comparable with that through the non-vulcanized filter
8 at the start of the flow measurement. However, at the end of the
9 measuring period, the K_s -value of the nonstabilized filter had
10 been reduced to a large extent which was not the case with the
11 vulcanized filter. After the flow measurement the filters were
12 sterilized through autoclaving of the filter (and the filter
13 apparatus) at 120°C for 20 minutes at the pressure 1.4 atm.
14 Flow measurements were carried out after the heat treatment and
15 the K_s -values are shown in Table 2..

16 No flow could be demonstrated through the nonstabilized
17 filter. On the other hand the vulcanized filter showed K_s -values
18 of the same order before as well as after autoclaving.

19 5 ml of a suspension of Sendai-virus were supplied to the
20 vulcanized filter. Filtration was carried out by means of a
21 water suction. When the liquid had passed the filter additional
22 5 ml of buffer solution were passed through the filter. This
23 was repeated twice. The filtrate was tested for hemagglutina-
24 tion activity: Inconsiderable hemagglutination activity could be
25 demonstrated. The upper surface of the filter was washed with
26 several portions of buffer solution. The washing liquid was
27 opalescent and its hemagglutination activity corresponded to a
28 yield of virus particles of almost 100%.

29 These examples show that the filters can be stabilized with
30 a dialdehyde such as glutar dialdehyde.

Type of filter	P ermeation Buffer.	Pressure dyn/cm ²	K _{S2} cm ²	Diameter μm	Ct, s	G el formation		pH	temp, °C
						fbg.konc.	$\frac{m}{2}$ g/1		
I a	Fibrin gel in +Ca ²⁺	TI	5.82x10 ⁻⁹	4.38 (0,258)	171	2.478	0.21	7.4	23
b	Vulcaniza- tion	"	-	5.20x10 ⁻⁹ (0,243)	"	"	"	"	"
c	Vulcaniza- tion and steri- lization	"	-	1.39x10 ⁻⁹ (0,126)	"	"	"	"	"
d	Control	"	-	3.15x10 ⁻⁹ (0,264)	"	"	"	"	"
II	Fibrin gel in cellulose sponge	TI, +Ca ²⁺	3430 0,61,0x10 ⁻¹¹	0,139-0,180 (0,008-0,011)	60	9.864	0.26	7.4	24
	Control	"	29400	1.14x10 ⁻¹¹ (0,011)	"	"	"	"	"
III a	Thin layer fibrin gel	TI +Ca ²⁺	7350 1.0-2,5x10 ⁻¹¹	0,180-0,284 (0,011-0,017)	60	9.864	0.26	7.4	24
b	Vulcaniza- tion	"	7350	3.6x10 ⁻¹¹ (0,020)	"	"	"	"	"
c	Vulcaniza- tion and sterilization	"	7350	2.24x10 ⁻¹¹ (0,016)	"	"	"	"	"
d	Vulcaniza- tion and sterilization	"	9,86x10 ⁵	3.52x10 ⁻¹² (0,0063)	0.107	"	"	"	"
e.	Vulcanization and sterilization	"	7350	1.66x10 ⁻¹¹ (0,014)	0.232	"	"	"	"
f	Control	"	29400	1.14x10 ⁻¹¹	0.192	60	9.864	0.26	7.40

TI = tris-imidazol buffer

See also explanation to Table I.

1 WHAT IS CLAIMED IS:

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2 1. A filter comprising fibrin in gel form, said
3 gel having substantially uniform pore sizes, said filter
4 comprising means for retaining the shape of at least one surface
5 of said gel against deformation when contacted by a flowing
6 medium.

7
8 2. A filter according to claim 1 wherein said
9 shape-retaining means comprises a foraminous sheet member.

10
11 3. A filter according to claim 2 wherein said
12 foraminous sheet member is in contact with said gel.

13
14 4. A filter according to claim 3 wherein said
15 foraminous sheet member comprises a fibrous network.

16
17 5. A filter according to claim 4 wherein said
18 network comprises a woven fabric.

19
20 6. A filter according to claim 4 wherein said
21 network comprises a knitted fabric.

22
23 7. A filter according to claim 4 wherein said
24 network comprises a non-woven fabric.

25
26 8. A filter according to claim 2 wherein said
27 shape-retaining means comprises a plurality of wires which
28 intersect one another.

1 9. A filter according to claim 8 wherein said
2 wires are in contact with said gel.

4 10. A filter according to claim 9 wherein said
5 wires are in the form of a wire screen.

7 11. A filter according to claim 9 wherein said
8 wires are in the form of a wire mesh.

10 12. A filter according to claim 9 wherein said wires
11 are in the form of an expanded wire sheet.

13 13. A filter according to claim 9 wherein said wires
14 are metal wires.

16 14. A filter according to claim 4 wherein said fib-
17 rous network comprises a sheet containing silk.

19 15. A filter according to claim 4 wherein said fib-
20 rous network comprises a sheet containing nylon.

22 16. A filter according to claim 4 wherein said fib-
23 rous network comprises a sheet containing cellulose.

25 17. A filter according to claim 4 wherein said fib-
26 rous network comprises a sheet containing cotton.

28 18. A filter according to claim 4 wherein said fib-
29 rous network comprises a sheet containing a natural fiber.

1 19. A filter according to claim 4 wherein said fib-
2 rous network comprises a sheet containing a synthetic fiber.

3
4 20. A filter according to claim 1 wherein the aver-
5 age ^{effective} pore size of said fibrin gel is 0,003 to 1,0 μm .

6
7 21. A filter according to claim 1 wherein the aver-
8 age ^{effective} pore size of said fibrin gel is 0,009 to 0,3 μm .

9
10 22. A filter according to claim 1 wherein said
11 fibrin gel is formed by contacting fibrinogen with an enzyme.

12
13 23. A filter according to claim 22 wherein said
14 enzyme is a coagulation enzyme.

15
16 24. A filter according to claim 23 wherein said
17 coagulation enzyme is selected from the group consisting of
18 thrombin, Batroxobin, Arvin, Eccarin, Papain, Staphylocoagulase,
19 Trypsin, caterpiller venom enzyme.

20
21 25. A filter according to claim 23 wherein said
22 fibrinogen is brought in contact with said enzyme in the pre-
23 sence of at least one calcium ion.

24
25 26. A filter according to claim 25 wherein the
26 calcium ion concentration in the gel formation mixture is up
27 to 20 mM.

1 27. A filter according to claim 23 wherein the
2 enzyme is thrombin and the gel is prepared in the absence of
3 a calcium ion.

4

5 28. A filter according to claim 25 wherein said
6 enzyme is Batroxobin.

7

8 29. A filter according to claim 1 wherein said
9 fibrin gel is a crosslinked fibrin gel and said shape-retaining
10 means is a crosslinking agent.

11

12 30. A filter according to claim 29 wherein said
13 crosslinking agent is selected from the group consisting of
14 dialdehydes, azides, aromaticdihalides, and bis-imidates.

15

16

17 31. A filter according to claim 29 wherein said
18 crosslinking agent is glutardialdehyde.

19

20 32. A filter according to claim 1 wherein said gel
21 is crosslinked and said shape-retaining means comprises a foram-
22 inous sheet member.

23

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25 33. A filter according to claim 32 wherein said
26 foraminous sheet member comprises a fibrous network.

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34. A filter according to claim 1 wherein said
gel has disposed on at least its upper surface a shape-retaining
means.

35. A filter according to claim 34 wherein said gel has disposed on its lower surface a second shape-retaining means.

36. A filter according to claim 35 wherein said second shape-retaining means comprises a forminous sheet.

37. A filter according to claim 1 wherein said gel is adhesively secured to said shape-retaining means.

38. A filter according to claim 1 wherein said gel is grafted to said shape-retaining means.

39. A filter according to claim 1 wherein said shape-retaining means is a foraminous member having pores or openings of a size of 0.01 to 5 mm.

40. A process for separating a first substance of 0,003 to 1 having a size/ μm from a second substance with which it is in admixture said second substance having a larger size than said first substance which comprises passing said mixture of said first substance and second substances over a filter comprising fibrin gel form having pores of substantially uniform size, said gel having pores of a size to permit passage therethrough of said first substance but excluding said second substance, said filter comprising means for retaining the shape of at least one surface of said gel against deformation when contacted by a flowing medium, wherein the effective pore size of said fibrin gel is larger than the particle size of said second substance.

41. A process according to claim 40 wherein said gel has substantially uniform pore sizes in the range of 0,009 to 0,3 μ m.

1 42. A process according to claim 40 wherein at
2 least one component of blood is separated from another com-
3 ponent by passing said blood over said filter.

4
5 43. A process according to claim 40 wherein at
6 least one component of blood plasma is removed from another
7 component by passing said vlood plasma over said filter.

8
9 44. A process according to claim 43 wherein
10 blood platelets are separated from blood plasma containing
11 blood platelets.

12
13 45. A process according to claim 40 wherein at
14 least one component of a mammalian liver is separated from
15 another component by passing the mixture over said filter.

16
17 46. A process according to claim 45 wherein
18 mitochondia is separated from liver cell fragnents.

19
20 47. A process according to claim 40 wherein a
21 virus is separated from components within which it is in ad-
22 mixture.

23
24 48. A process according to claim 47 wherein said
25 virus is Sendai-virus.

26
27 49. A process according to claim 40 wherein a
28 bacterium is separated from components within which it is in
29 admixture.

1 50. A process according to claim 49 wherein said
2 bacterium is *E. coli*.

3
4 51. A process according to claim 22 wherein said
5 gel is formed in the presence of Factor XIII.

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FIG. 1a.

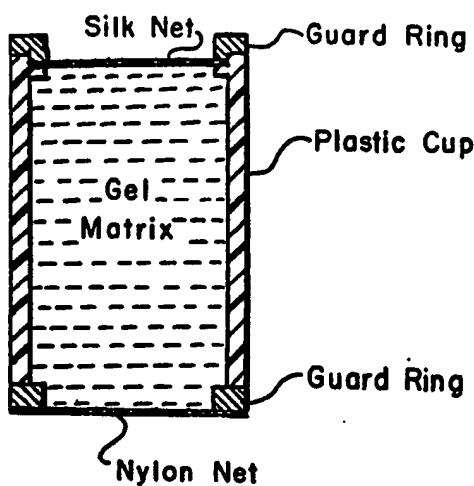


FIG. 1b.

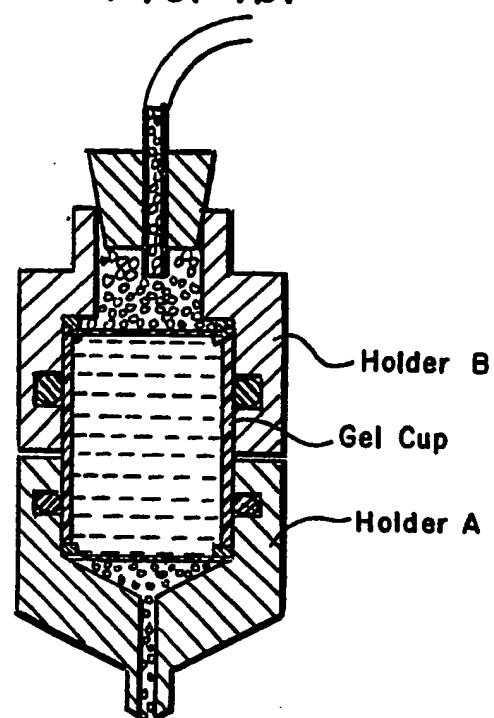


FIG. 8a.

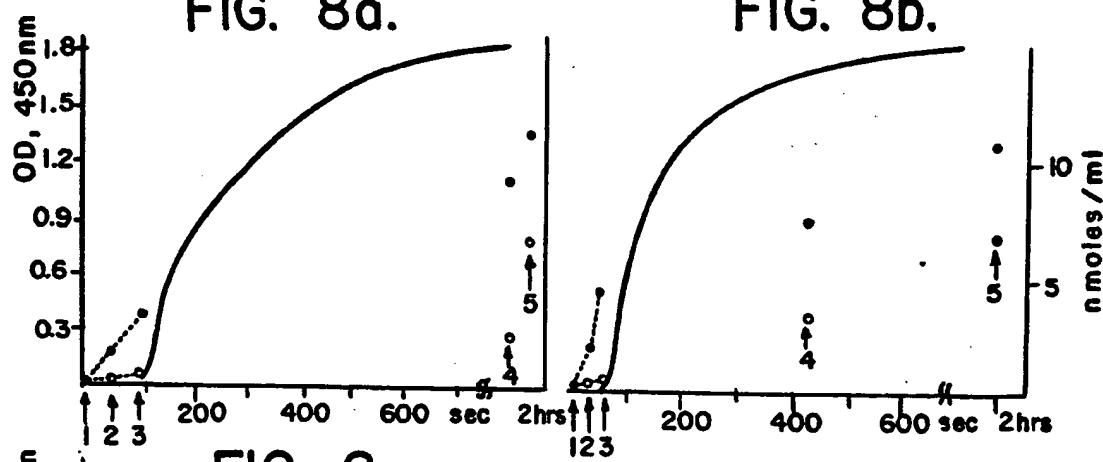


FIG. 8b.

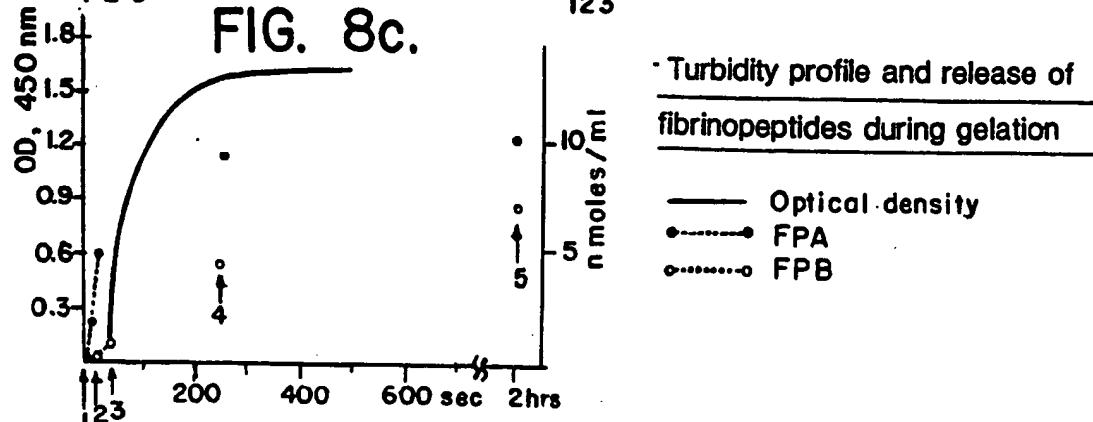


FIG. 8c.

Turbidity profile and release of
fibrinopeptides during gelation

— Optical density
● FPA
○ FPB

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FIG. 2b.

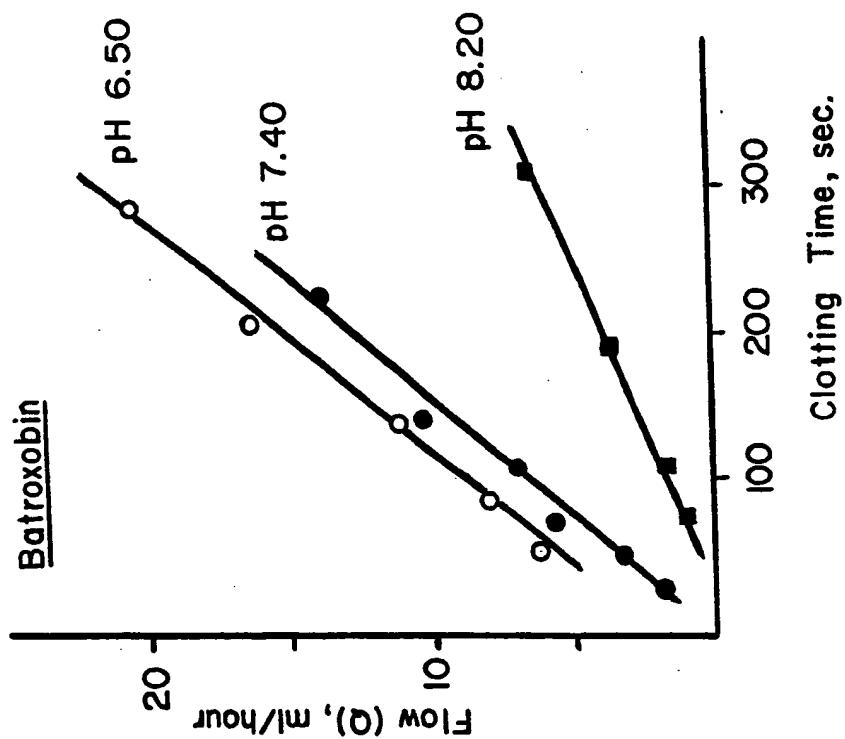
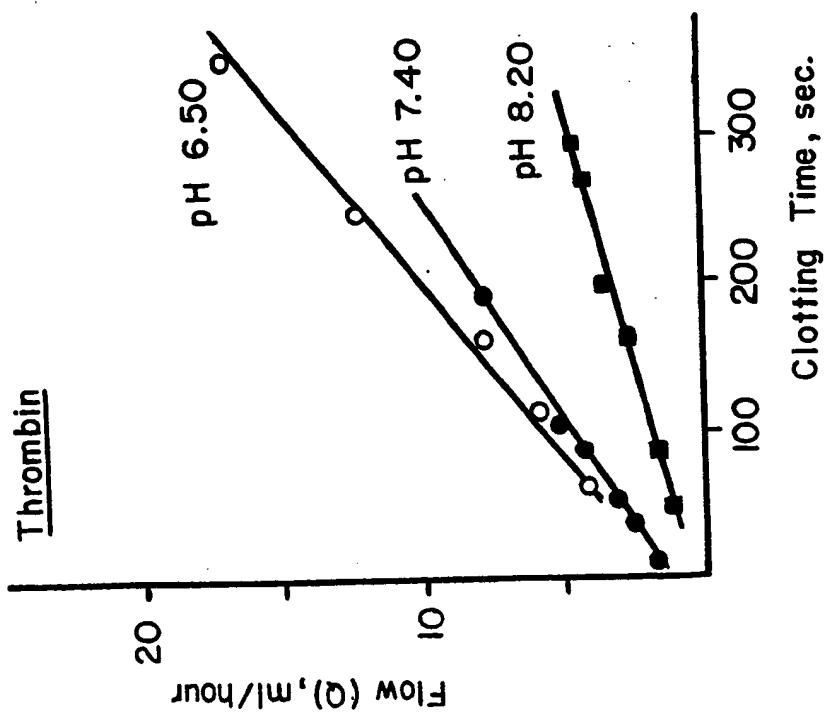


FIG. 2a.



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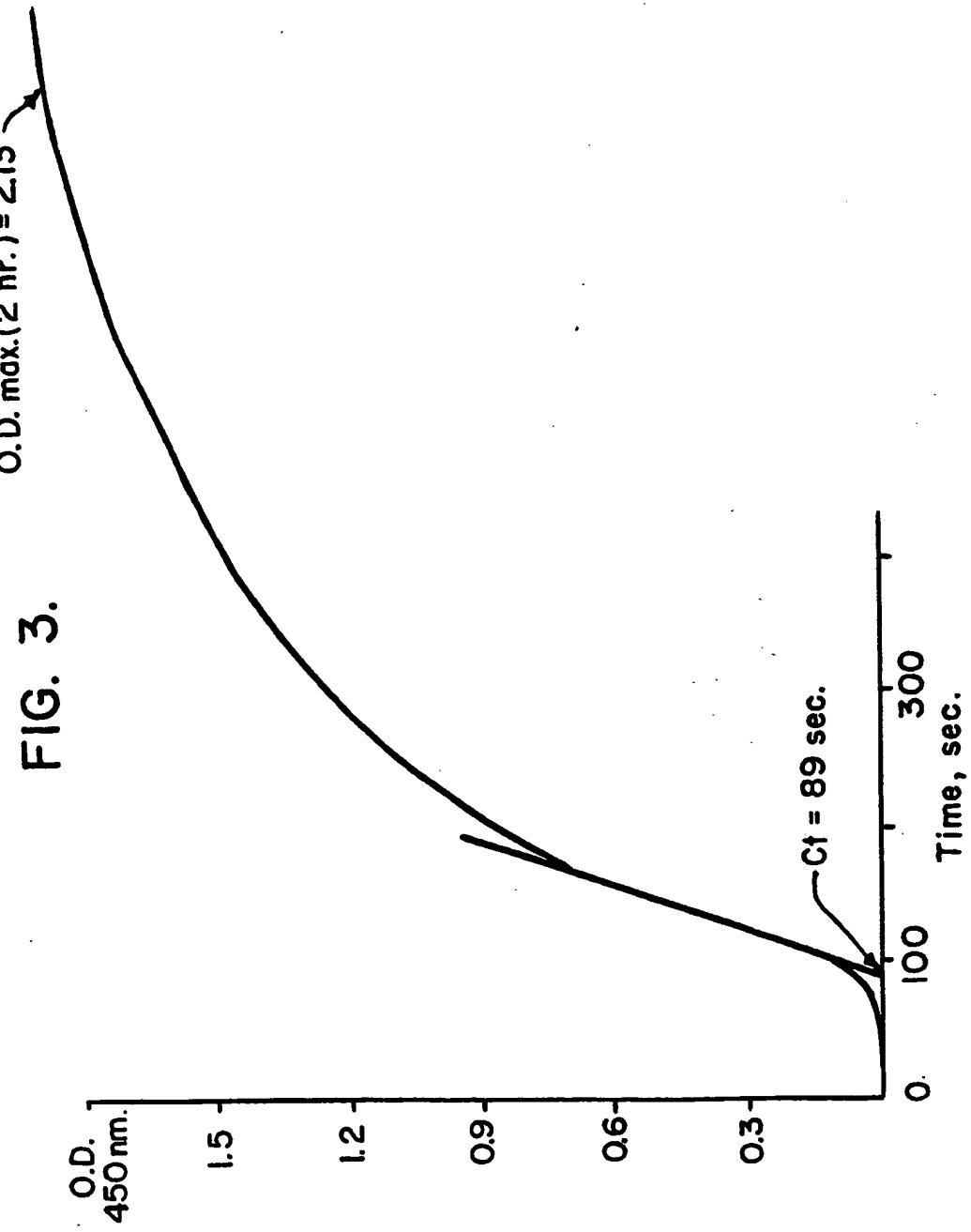


FIG. 4a.

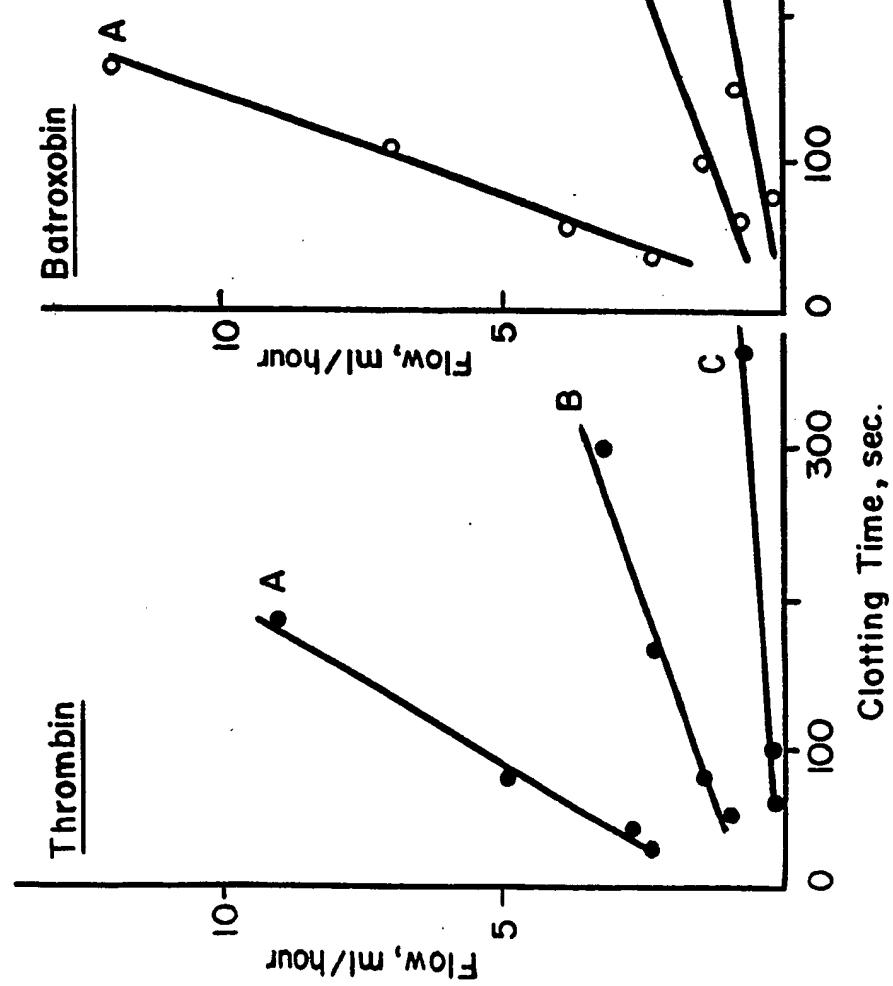
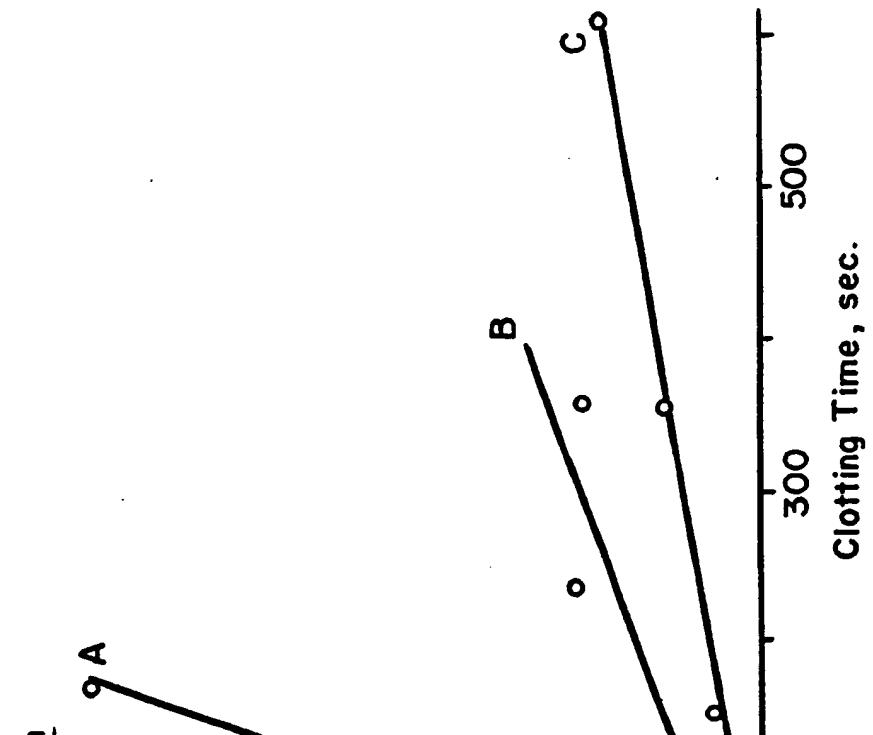


FIG. 4b.



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FIG. 5b.

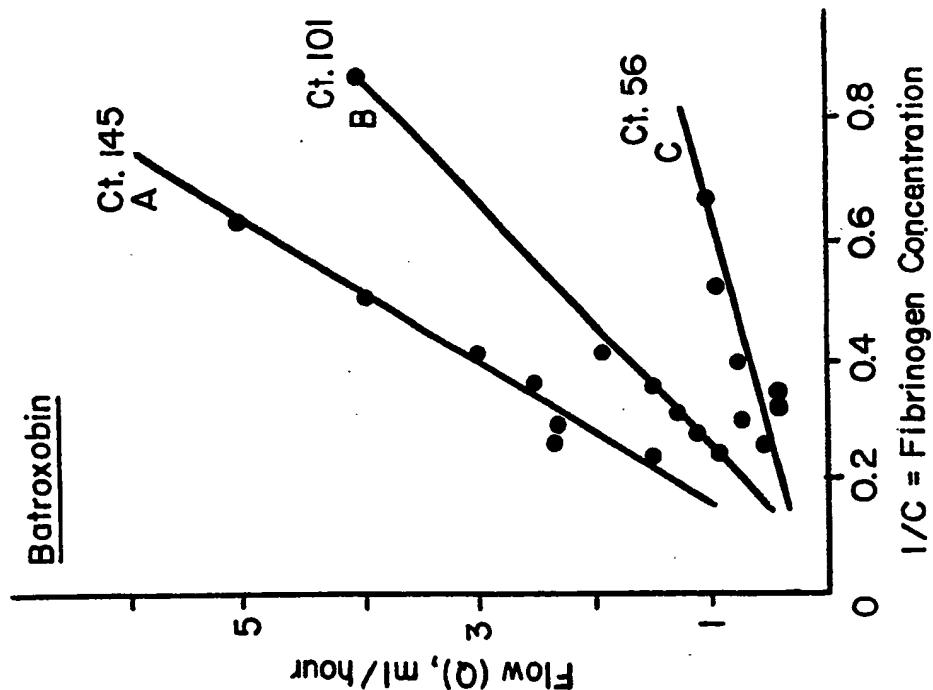
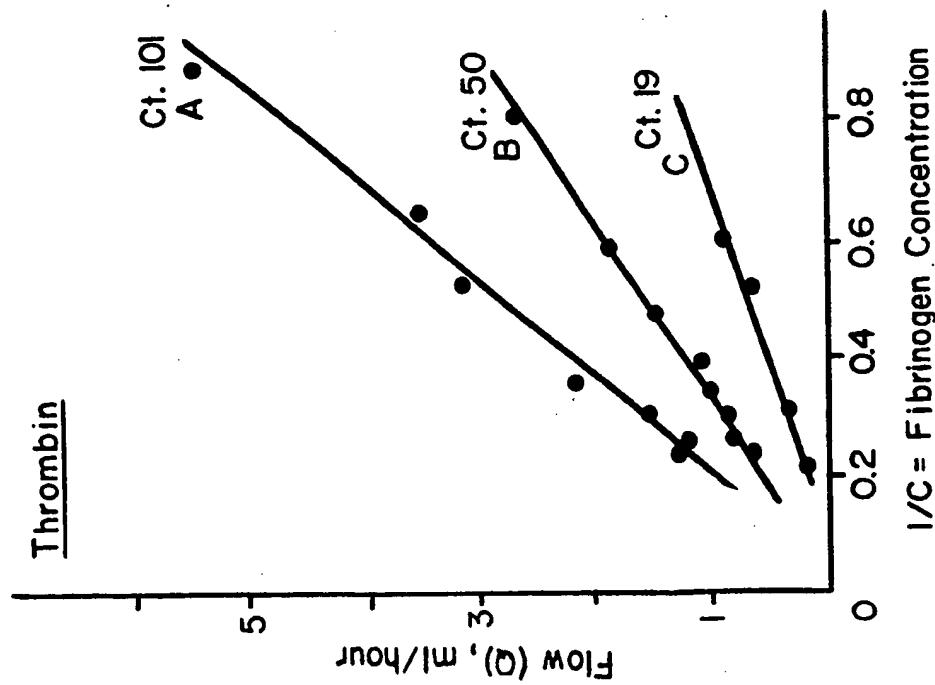


FIG. 5a.



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FIG. 6b.

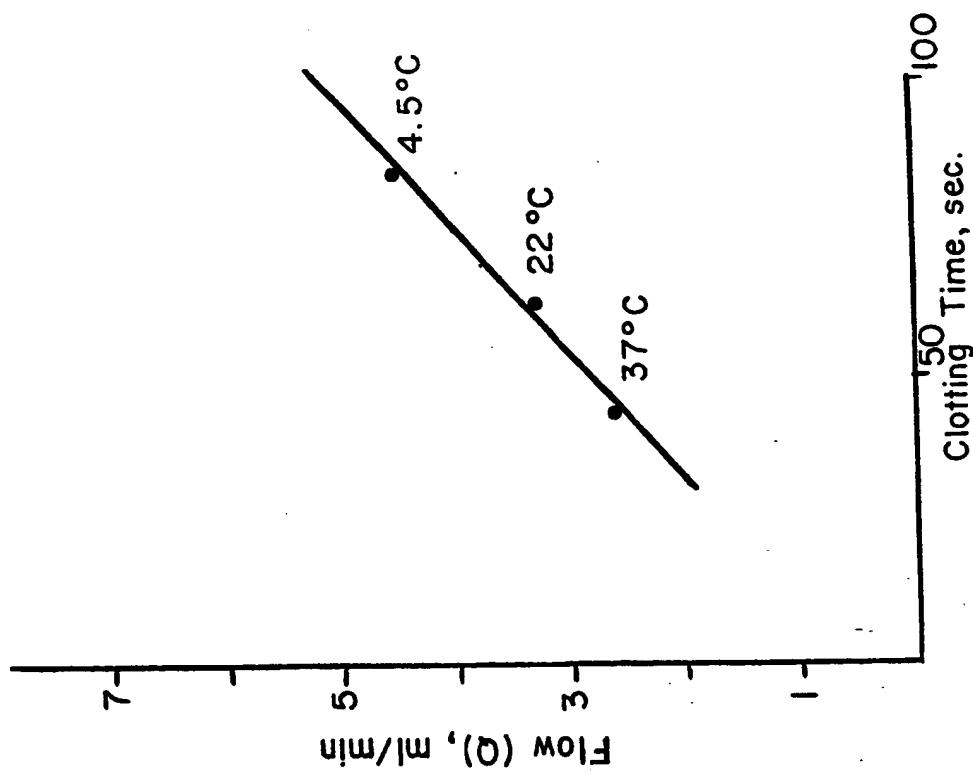
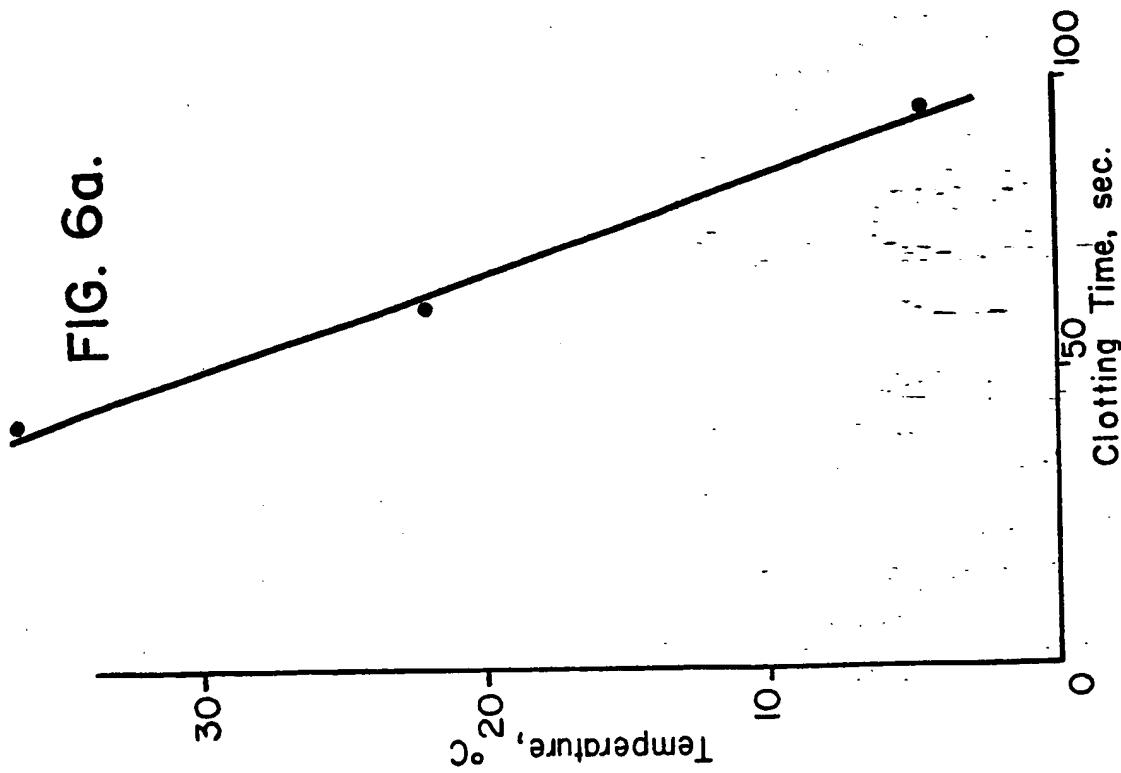


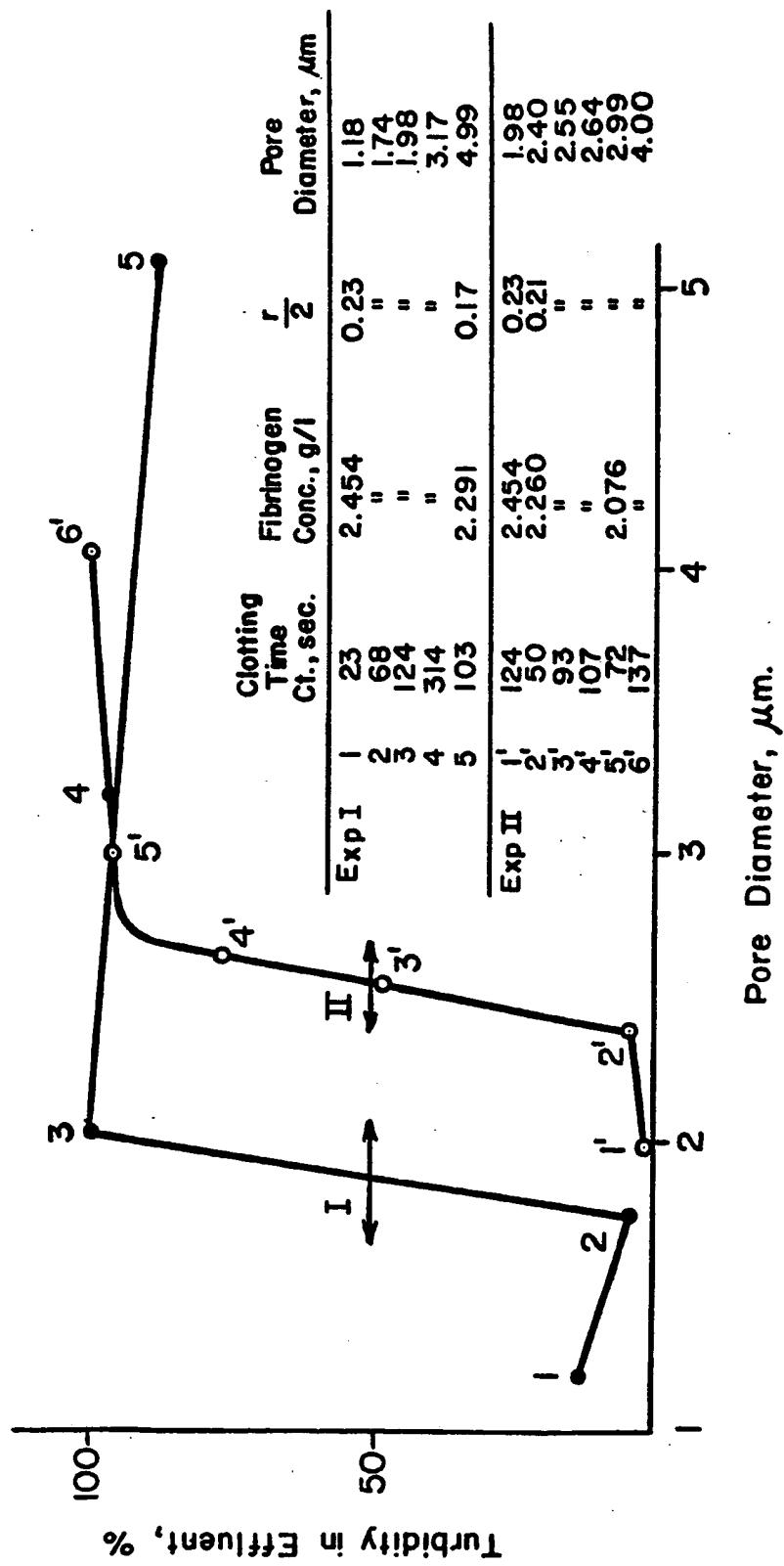
FIG. 6a.



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FIG. 7.





European Patent
Office

EUROPEAN SEARCH REPORT

0085166

Application number

EP 82 11 1629

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int. Cl. *)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
A	DE-A-2 009 515 (M. PETROW) * Claim *	1	B 01 D 13/04 B 01 D 31/00
A	---	1	
A	DE-A-2 025 649 (UNITED STATES BANKNOTE CORP.) * Claims 1, 10, 20, page 1, paragraph 2 - page 2, paragraph 3 *		
A	---		
A	DE-A-2 432 049 (C.H. BOEHRINGER SOHN) * Claims 1-4, 7, 8 *	1-4, , 16	
A	---	1	
A	DE-B-2 407 961 (W.R. VIETH et al.) * Claim 1 *		
A	---		
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The present search report has been drawn up for all claims			
Place of search BERLIN	Date of completion of the search 22-03-1983	Examiner KUEHN P	
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DOCUMENTS CONSIDERED TO BE RELEVANT			Page 2
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.)
A	Chemical Abstracts vol. 88, no. 17, 24 April 1978 Columbus, Ohio, USA page 328, column 1, Abstract no. 126372h & JP-A-77-156912 ---	1,22-25	
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			TECHNICAL FIELDS SEARCHED (Int. Cl.)
<p>The present search report has been drawn up for all claims</p>			
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